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48.—REPORT OF THE IRISH RADIUM COMMITTEE FOR THE YEAR 1936. (Including Reports from St. Anne's Hospital, Dublin (*Radiologist*—DR. OLIVER CHANCE), and by OLIVER CHANCE, M.B., B.CH., D.M.R.E., Richmond, Sir Patrick Dun's, Dr. Steevens', and Coombe Hospitals, and OSWALD J. MURPHY, M.B., B.CH., St. Vincent's Hospital, Dublin.)

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[*Authors alone are responsible for all opinions expressed in their Communications.*]

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No. 48.

REPORT OF THE IRISH RADIUM COMMITTEE FOR THE YEAR 1936.

[Read MAY 25. Published separately JUNE 30, 1937.]

1,793 tubes containing 12,635 millicuries of radon were issued for therapeutic purposes during the year, compared with 2,540 tubes containing 16,581 mc. issued during 1935, and with 2,312 tubes containing 11,744 mc. in 1934. The total issued is thus intermediate between those for the two previous years, while the average activity per tube has risen from 5·1 mc. in 1934 to 6·5 mc. in 1935 and to 7·0 mc. in 1936. It is difficult to give useful results for the number of millicuries used per patient, since the treatment of a number of the patients referred to in the reports received was carried out with solid radium, and not with radon supplied by the Committee.

No important changes have been made in the technique of the preparation of radon tubes. Dr. P. Fitzgerald and Dr. F. O. Pilkington were elected to the Radium Exhibitions falling vacant during the year.

In addition to the cases enumerated in the following reports, returns of numbers of cases treated have been received from the following hospitals:—

Central Hospital, Galway	... ...	19 cases.
Coombe Hospital, Dublin	... ...	6 „
Dr. Steevens' Hospital, Dublin	... ...	2 „

The total number of cases treated with radium or radon of which reports have been received was—

	1935.	1936.
Malignant	451	453
Non-malignant	149	99
Total, ...	600	552

It is probable that in 1936 a larger proportion of cases were treated with radium rather than radon, as compared with previous years. Thus, for 222 out of the 329 treatments carried out at St. Anne's Hospital radium element was used.

REPORT FROM ST. ANNE'S HOSPITAL, DUBLIN.

The number of malignant cases treated in St. Anne's continues to increase. During 1936, 95 more malignant cases were admitted than in the previous year. A very much larger proportional increase occurred in the number of cases returning for routine examination. This is the direct result of the adoption of an efficient "follow-up" system. While it is gratifying to know that our efforts to examine all patients periodically are appreciated, the success of the follow-up system is actually the cause of acute embarrassment, for it has thrown a greatly increased strain on our accommodation.

Technique.

In this report it is not proposed to enter into details of technique. (It is hoped that in each future annual report a single form of cancer will be chosen and its treatment by radiation discussed in detail.) Owing to lack of facilities it has been found impossible to standardise techniques. We can, however, note definite trends. Surface therapy is being used more and more to the exclusion of implantation in most skin and lip cancers, and many oral neoplasms. Various substances are used as moulds. We find that in most cases felt (covered with dental wax if discharge is present) makes the best and simplest mould in skin cases. Stent or base plate is usually used in the mouth or on lips. All growths are very carefully measured, and dosage is calculated according to the principles of Paterson and Parker. We feel sure that radium element is better than radon in surface therapy, and its use is always preferred when moulds are used.

Where implantation is employed, it is carefully controlled by calculation and mensuration. At present we prefer X-ray therapy to radium in the treatment of advanced tumours of the mouth. During the past six months, with improved techniques, surprising immediate results have been obtained in advanced cases.

As in the case of radium applications, all treatments carried out by X-rays are carefully planned and checked by means of Holdfelder's Atlas, and measurements in a water phantom. Furthermore, no opportunity of checking dosage with a mekapion dosimeter during treatment is ever lost.

"Follow-up" System.

This is one of the most important departments of a Cancer Clinic. The routine used in St. Anne's is as follows:—Every patient, when leaving hospital, is given a card on which is written the date on which he is due to return for re-examination. (This is usually 4–12 weeks after the date of discharge.) A companion card is placed on a date file, and, if necessary, a bed is booked for that date. The patient's doctor is also written to, the nature of the treatment is explained, and the possible sequelae discussed. In the case of County Board cases, the Co. Medical Officer must also be written to. Should he fail to attend on the appointed day, we get into touch with the patient, and, if necessary, his doctor, so that an explanation is obtained.

The follow-up system entails a very considerable amount of correspondence. During the past two months over 1,500 letters have been circulated in connection with this system. It will be noted from tables that we have actually traced over 90 per cent. of the patients treated during the last three years. These figures compare more than favourably with those obtained at old-established centres equipped with fully-staffed "follow-up" and almoner's departments. Our success is largely due to the patient himself, and the co-operation of both country doctors and Co. Medical Officers, who show very great interest in the welfare of their patients; and frequently they have gone to considerable trouble to keep us informed about a patient who has failed to report regularly.

Another result of our system has been that many doctors have become acquainted with the fundamentals upon which the modern treatment of cancer is based. We feel that the education of the doctor in such a manner is necessary before any attempt at anti-cancer propaganda can be applied to the public.

The following up of patients is associated with two difficulties, neither of which is a fault of the system.

Firstly, patients from distant counties are often brought to hospital by an ambulance which returns immediately to the country. When examined it is found that many of these patients do not require further treatment. However, they must stay in the hospital, and occupy a badly-needed bed until the ambulance returns for them, possibly not until more than a week has elapsed.

Our other difficulty lies in persuading the poor country patient who does not receive State help to travel periodically to Dublin. Their unwillingness cannot be wondered at, when it is realised that a return ticket to Dublin from parts of Ireland may cost up to £4. In order to get over these difficulties we are considering holding periodic clinics in the different counties.

Radium.

No. of Treatments:—			
Radium—			
New Cases	186		
Return Cases	36		
	—	222	
Radon—			
New Cases	95		
Return Cases	12		
	—	107	
	Total,	329	

Malignant Cases, 1936. New Cases. All Types.

No. of cases of all types of malignant disease seen, 510.
No. unsuitable for treatment, 92.
No. refused treatment, 9.
No. of cases treated, 409.

By Surgery alone	27
„ Radium alone	208
„ Deep X-ray alone	137
„ Radium and Surgery	9
„ Deep X-ray and Surgery	9
„ Radium and X-rays	13
„ Radium, Surgery, and X-rays	2
„ Radium and Superficial X-rays	1
„ Superficial X-rays alone	3
Total,	409

MALIGNANT CASES, 1936.

Malignant Cases, 1936.	No. Cases seen.	Unsuitable for treatment.	Refused treatment.	No. Cases treated.	Radium alone.	Deep X-Rays alone.	Surgery alone.	Superficial X-Ray alone.	Radium and Surgery.	Radium and X-Rays.	Surgery and X-Rays.	Radium, Surgery, and X-Rays.	Radium and Superficial Therapy.	RESULTS.	Free from Disease.	Still under treatment.	Unsuitable further treatment.	Dead.	Untraced.
Rodent Ulcers ...	57	—	—	57	53	—	1	2	—	—	—	—	1		36	10	2	2	7
Carcinoma Skin ...	54	5	3	46	35	5	4	1	1	—	—	—	—		22	14	5	—	5
,, Tongue ...	53	17	1	35	21	7	2	—	2	2	—	1	—		19	3	5	2	6
,, Lip ...	72	12	—	60	51	2	1	—	3	1	1	1	—		33	10	7	2	8
,, Floor Mouth	13	3	—	10	7	1	—	—	1	1	—	—	—		4	—	6	—	—
,, Gum ...	6	2	—	4	—	4	—	—	—	—	—	—	—		—	1	3	—	—
,, Cheek ...	7	2	—	5	5	—	—	—	—	—	—	—	—		2	1	1	—	1
,, Tonsil ...	10	—	—	10	—	9	—	—	—	1	—	—	—		2	3	2	2	1
,, Fauces ...	7	1	—	6	1	3	1	—	—	1	—	—	—		2	—	2	—	—
,, Palate ...	9	2	—	7	4	3	—	—	—	—	—	—	—		1	—	4	1	1
,, Naso-Pharynx	1	—	—	1	—	1	—	—	—	—	—	—	—		—	1	—	—	—
,, Epiglottis ...	3	—	—	3	—	3	—	—	—	—	—	—	—		—	—	2	—	1
,, Larynx ...	20	2	1	17	—	16	1	—	—	—	—	—	—		6	—	2	6	3
,, Pharynx ...	4	1	—	3	—	2	—	—	—	—	1	—	—		—	—	1	2	—
,, Ear ...	1	—	—	1	—	1	—	—	—	—	—	—	—		—	—	—	1	—
,, Nose ...	1	—	—	1	1	—	—	—	—	—	—	—	—		1	—	—	—	—
,, Branchial ...	3	1	1	1	—	1	—	—	—	—	—	—	—		—	—	—	1	—
,, Pr. Glands Nk.	8	2	—	6	—	5	1	—	—	—	—	—	—		3	—	3	—	—
,, Max. Antrum	6	1	—	5	—	5	—	—	—	—	—	—	—		1	—	2	1	1
,, Parotid Gld. ...	4	—	—	4	2	2	—	—	—	—	—	—	—		—	1	—	2	1
,, Breast ...	54	11	1	42	6	21	4	—	1	5	5	—	—		11	5	18	4	4
,, Oesophagus ...	6	1	—	5	—	1	3	—	1	—	—	—	—		—	—	4	1	—
,, Stomach ...	5	5	—	—	—	—	—	—	—	—	—	—	—		—	—	—	—	—
,, Bladder ...	1	1	—	—	—	—	—	—	—	—	—	—	—		—	—	—	—	—
,, Body Uterus ...	10	2	1	7	1	6	—	—	—	—	—	—	—		—	2	—	—	5
,, Cervix Uteri ..	26	2	1	23	14	6	1	—	—	2	—	—	—		9	4	3	5	2
,, Ovary ...	2	—	—	2	—	2	—	—	—	—	—	—	—		—	—	—	—	2
,, Vulva ...	5	2	—	3	3	—	—	—	—	—	—	—	—		1	—	2	—	—
,, Vagina ...	1	—	—	1	1	—	—	—	—	—	—	—	—		1	—	—	—	—
,, Rectum ...	14	6	—	8	—	1	7	—	—	—	—	—	—		1	—	5	2	—
,, Anus ...	1	1	—	—	—	—	—	—	—	—	—	—	—		—	—	—	—	—
,, Gland Groin ...	1	—	—	1	—	—	—	—	—	—	1	—	—		—	—	1	—	—
,, Testes ..	2	—	—	2	—	2	—	—	—	—	—	—	—		—	—	2	—	—
,, Prostate ...	1	1	—	—	—	—	—	—	—	—	—	—	—		—	—	—	—	—
,, Penis ...	2	1	—	1	1	—	—	—	—	—	—	—	—		1	—	—	—	—
,, Uvula ...	1	—	—	1	—	1	—	—	—	—	—	—	—		—	—	1	—	—
Sarcoma ...	19	4	—	15	1	14	—	—	—	—	—	—	—		—	2	5	4	4
,, Melanotic ...	1	—	—	1	1	—	—	—	—	—	—	—	—		—	—	—	—	1
Mediastinal Tumour	1	—	—	1	—	1	—	—	—	—	—	—	—		—	—	—	1	—
Pituitary Tumour ...	1	—	—	1	—	1	—	—	—	—	—	—	—		—	—	1	—	—
Hodgkin's Disease ...	9	—	—	9	—	9	—	—	—	—	—	—	—		—	—	—	—	9
Paget's Disease ...	2	1	—	1	—	1	—	—	—	—	—	—	—		—	—	—	—	1
Malignant Ovarian Cyst ...	4	2	—	2	—	1	1	—	—	—	—	—	—		—	—	—	—	2
Endothelioma Shoulder ...	1	1	—	—	—	—	—	—	—	—	—	—	—		—	—	—	—	—
Malignant Polypus Nostril ..	1	—	—	1	—	—	—	—	—	—	1	—	—		—	—	1	—	—
TOTALS, ... (Including 46 Out-Patients.)	510	92	9	409	208	137	27	3	9	13	9	2	1		156	57	90	39	67

Non-Malignant Cases treated with Radium or Radon, 1936.

Naevi Flammens	3	Toxic Goitre	1
Verucae Vulgaris	2	Septic Granuloma Larynx ...	1
T. B. Peritonitis	7		—
		Total,	14

RECORD OF CASES TREATED WITH RADIUM OR RADON IN PAST THREE YEARS.

Cancer of Lip.

Stage I.

Year.	Treated.	Alive years after Treatment.		
		1	2	3
1934	11	8	7	6
1935	6	6	5	
1936	35	24		

Stage II.

Year.	Treated.	Alive years after Treatment.		
		1	2	
1934	2	–	–	–
1935	6	3	3	
1936	7	7		

Stage III.

Year.	Treated.	Alive years after Treatment.		
		1	2	3
1934	4	1	1	1
1935	16	9	8	
1936	16	13		

Cancer of Tongue.

Stage I.

Year.	Treated.	Alive years after Treatment.		
		1	2	3
1934	10	7	5	3
1935	20	13	10	3
1936	20	14		

Stage II.

Year.	Treated.	Alive years after Treatment.		
		1	2	
1934	5	2	2	1
1935	8	2	1	
1936	9	8		

Stage III.

Year.	Treated.	Alive years after Treatment.		
		1	2	3
1934	3	–	–	–
1935	11	1	1	
1936	5	2		

Cancer of Floor of Mouth.

Stage I.

Year.	Treated.	Alive years after Treatment.		
		1	2	3
1934	3	2	2	2
1935	2	2	2	
1936	7	7		

Stage II.

Year.	Treated.	Alive years after Treatment.		
		1	2	3
1934	–	–	–	–
1935	1	1		
1936	–			

Stage III.

Year.	Treated.	Alive years after Treatment.		
		1	2	3
1934	–	–	–	–
1935	5	1	–	
1936	3	3		

Cancer of Cheek.

Stage I.

Year.	Treated.	Alive years after Treatment.		
		1	2	3
1934	1	1	1	1
1935	5	4	3	
1936	3	1		

Stage II.

Year.	Treated.	Alive years after Treatment.		
		1	2	3
1934	1	–	–	–
1935	0	–	–	
1936	2	2		

Stage III.

Year.	Treated.	Alive years after Treatment.		
		1	2	3
1934	2	–	–	–
1935	1	–	–	
1936	–	–		

Cancer of Gum.

Stage I.

Year.	Treated.	Alive years after Treatment.		
		1	2	3
1934	–	–	–	–
1935	3	–	–	–
1936	–	–		

Stage II.

Year.	Treated.	Alive years after Treatment.		
		1	2	3
1934	–	–	–	–
1935	–	–	–	
1936	1	1		

Stage III.

Year.	Treated.	Alive years after Treatment.		
		1	2	3
1934	2	–	–	–
1935	4	–	–	
1936	3	3		

Cancer of Palate.

Stage I.

Year.	Treated.	Alive years after Treatment.		
		1	2	3
1934	1	1	1	1
1935	3	1	–	
1936	3	2		

Stage II.

Year.	Treated.	Alive years after Treatment.		
		1	2	3
1934	–	–	–	–
1935	–	–	–	
1936	4	3		

Stage III.

Year.	Treated.	Alive years after Treatment.		
		1	2	3
1934	1	–	–	–
1935	2	–	–	
1936	–	–		

Cancer of Fauces.

Stage I.

Year.	Treated.	Alive years after Treatment.		
		1	2	3
1934	–	–	–	–
1935	2	–	–	
1936	1	1		

Stage II.

Year.	Treated.	Alive years after Treatment.		
		1	2	3
1934	–	–	–	–
1935	–	–	–	
1936	2	2		

Stage III.

Year.	Treated.	Alive years after Treatment.		
		1	2	3
1934	–	–	–	–
1935	–	–	–	
1936	3	2		

Cancer of Tonsil.

Stage I.

Year.	Treated.	Alive years after Treatment.		
		1	2	3
1934	–	–	–	–
1935	4	3	3	
1936	2	2		

Stage II.

Year.	Treated.	Alive years after Treatment.		
		1	2	3
1934	–	–	–	–
1935	1	1	1	
1936	–	–		

Stage III.

Year.	Treated.	Alive years after Treatment.		
		1	2	3
1934	4	2	–	–
1935	9	3	2	
1936	8	3		

Cancer of Skin.

Stage I.

Year.	Treated.	Alive years after Treatment.		
		1	2	3
1934	19	13	13	13
1935	38	36	27	
1936	40	39		

Stage II.

Year.	Treated.	Alive years after Treatment.		
		1	2	3
1934	–	–	–	–
1935	1	1	1	
1936	2	2		

Stage III.

Year.	Treated.	Alive years after Treatment.		
		1	2	3
1934	–	–	–	–
1935	1	1	1	
1936	1	1		

Cancer of Cervix Uteri.

Stage I.

Year.	Treated.	Alive years after Treatment.		
		1	2	3
1934	1	1	1	1
1935	0	–	–	
1936	2	2		

Stage II.

Year.	Treated.	Alive years after Treatment.		
		1	2	3
1934	1	1	1	1
1935	5	3	2	
1936	4	3		

Stage III.

Year.	Treated.	Alive years after Treatment.		
		1	2	3
1934	–	–	–	–
1935	5	–	–	
1936	16	12		

Rodent Ulcers.

Year.	Treated.	Alive years after Treatment.		
		1	2	3
1934	49	31	23	17
1935	38	32	31	
1936	57	48		

N.B.—All patients untraced are regarded as dead. In several such cases we have reason to believe that the patients are actually alive and free from disease.

Report by Oliver Chance, M.B., B.Ch., D.M.R.E.,

Richmond, Sir P. Dun's, Dr. Steevens', Rotunda, and Coombe Hospitals.

During 1936 most of the cases requiring radiation seen in the above hospitals were referred to St. Anne's Hospital for treatment. The bulk of the cases included below were either private patients or else suffering from such conditions as Rodent Ulcers for which they could be treated as out-patients.

Malignant Cases (treated with Radium or Radon), 31.

Rodent Ulcers, 14.

Treated by surgery, 1. Treated with radium, 12. Well, 11. Untraced, 1. Unsuitable, 1.

Carcinoma of Skin, 5.

Unsuitable, 3. Well, 1. Untraced, 1.

Carcinoma Ovary, 3.

Post-Operative X-rays, 3. Well, 1. Dead, 2.

Carcinoma Breast, 5.

(*a*) Operable, 2. Post-Operative Prophylactic X-rays, 2. Well, 2.

(*b*) Inoperable, 3. Treated with X-rays, 3. Improved, 1. Untraced, 1. Dead, 1.

Branchial Carcinoma, 1.

Treated with X-rays, 1. Dead, 1.

Sarcoma, 3.

Treated with X-rays, 3. Well, 1. Unimproved, 1. Dead, 1.

	No. of cases.	Treated.	Well.	Primary well, but glands involved.	Untraced.	Dead.
Carcinoma Lip, 2.						
	2	2	1	–	1	–
Carcinoma Tongue, 6.						
Group 1 ...	–	–	–	–	–	–
Group 2 ...	3	3	1	1	–	1
Group 3 ...	3	2	–	1	1	–
Carcinoma Cheek, 1.						
Group 2 ...	1	–	–	–	–	1
Carcinoma Larynx, 3.						
(Treated with X-rays)						
Group 1 ...	2	2	1	–	1	–
Group 2 ...	1	–	–	–	–	1
Group 3 ...	–	–	–	–	–	–
Post-cricoid Carcinoma.						
(Treated with X-rays)	1	1	–	–	–	1
Carcinoma Cervix, 8.						
Group 1 ...	1	1	1	–	–	–
Group 2 ...	4	4	3	–	–	1
Group 3 ...	3	3	1	–	1	1

Carcinoma Prostate, 2.
Treated with X-rays, 2. Improved, 2.

Carcinoma Bladder, 1.
Treated with X-rays, 1. Unimproved, 1.

Carcinoma Penis, 1.
Treated with radium, 1. Well, 1.

Carcinoma Parotid, 1.
Treated with radium, 1. Well, 1.

Carcinoma Rectum, 3.
Unsuitable, 3.

Hodgkin's Disease, 1.
Treated with X-rays, 1. Untraced, 1.

Non-Malignant Cases (treated with Radium or Radon), 22.

Menopausal Hæmorrhage, 2.
Well, 2.

Toxic Goitre, 11.
Well, 1. Improved, 6. Untraced, 4.

Tubercular Enteritis, 9.
Well, 2. Improved, 1. Untraced, 4. Dead, 2.

Report by Dr. Oswald J. Murphy,
St. Vincent's Hospital, Dublin.

Cases seen and treated with radium or radon during the year 1936.

Malignant cases treated by surgery alone	0
Malignant cases treated by surgery and radium	21
Malignant cases treated by radium alone	92
Malignant cases treated by radium and X-ray	1

Malignant Cases (114).

	Seen.	Treated.
Carcinoma of the Ear ...	5	5
„ of the Lip ...	8	8
„ of the Cheek ...	3	3
„ of the Tongue ...	10	10
„ of Floor of Mouth	1	1
„ of Palate ...	1	1
„ of Tonsil ...	2	1
„ of Pharynx ...	1	–
„ of Oesophagus ...	2	–
„ of Breast ...	4	4
„ of Breast (Secondaries) ...	8	8
„ of Penis ...	3	2
„ of Anus ...	1	1
„ of Uterine Cervix ...	7	7
„ of Uterine Body ...	5	4
„ of Skin Surfaces	22	20
Rodent Ulcers	38	38
Sarcoma of Cheek	1	1
	122	114

Non-Malignant Cases (77).

	Treated.
Goitre	5
Hodgkin's Disease	4
T.B. Glands	9
T.B. Peritonitis	4
Uterine Hæmorrhages	4
Angiomata	14
Lupus Vulgaris	2
Lupus Erythematosis	2
Keloids	3
Dupytron	6
Warts	21
Hypertrichosis	3
	77

Results of Treatment.

Malignant Cases.

Carcinoma of the Ear, 5.

	No. treated.	Apparently well.	Improved.	Unimproved.	Untraced.	Died.
No glands ...	5	2	2	1	–	–
Glands ...	–	–	–	–	–	–

Carcinoma of the Lip, 8.

	No. treated.	Apparently well.	Improved.	Unimproved.	Untraced.	Died.
No glands ...	7	4	2	–	1	–
Glands ...	1	–	–	1	–	–

Carcinoma of the Cheek, 3.

	No. treated.	Apparently well.	Improved.	Unimproved.	Untraced.	Died.
No glands ...	2	1	1	–	–	–
Glands ...	1	–	–	1	–	–

Carcinoma of the Tongue, 7.

Anterior Third of Tongue, 1.

	No. treated.	Apparently well.	Improved.	Unimproved.	Untraced.	Died.
No glands ...	1	1	–	–	–	–
Glands ...	–	–	–	–	–	–

Middle Third, 3.

No glands ...	1	1	–	–	–	–
Glands ...	2	–	1	1	–	–

Posterior Third, 3.

No glands ...	–	–	–	–	–	–
Glands ...	3	1	1	–	–	1

Carcinoma of Floor of Mouth, 1.

	No. treated.	Apparently well.	Improved.	Unimproved.	Untraced.	Died.
No glands ...	1	1	–	–	–	–
Glands ...	–	–	–	–	–	–

Carcinoma of Hard Palate, 1.

An early case, no glands, and is now apparently cured.

Carcinoma of Tonsil.

One case was in no state to stand radium treatment and was hopeless. The second case is still under treatment.

Carcinoma of Breast (Primary), 4.

	No. treated.	Apparently well.	Improved.	Unimproved.	Untraced.	Died.
No glands ...	3	2	1	–	–	–
Glands ...	1	–	–	–	1	–

Carcinoma of Breast (Secondaries), 8.

No. treated.	Apparently well.	Improved.	Unimproved.	Untraced.	Died.
8	1	3	2	1	1

Carcinoma of the Penis, 2.

Two early cases are progressing very favourably, but the third was referred to a surgeon.

Carcinoma of Uterine Cervix, 7.

	No. treated.	Apparently well.	Improved.	Unimproved.	Untraced.	Died.
Group I ...	1	1	–	–	–	–
Group II ...	2	1	–	–	1	–
Group III ...	4	1	1	–	–	2

Carcinoma of Uterine Body, 4.

	No. treated.	Apparently well.	Improved.	Unimproved.	Untraced.	Died.
Group I ...	–	–	–	–	–	–
Group II ...	2	1	1	–	–	–
Group III ...	2	–	1	–	–	1

These four cases refused operation.

Carcinoma of Skin Surface, 20.

	No. treated.	Apparently well.	Improved.	Unimproved.	Untraced.	Died.
No glands ...	16	12	2	–	2	–
Glands ...	4	1	2	1	–	–

Rodent Ulcers, 38.

No. treated.	Apparently well.	Improved.	Unimproved.	Untraced.	Died.
38	32	3	1	2	–

Sarcoma of the Cheek, 1.

This is a case with secondary extension from a scar. The primary had been removed surgically.

Non-Malignant Cases, 77.

Hodgkin's Disease, 4.

All four cases have reacted well locally, and one shows no signs of recurrence after 10 months.

Tuberculous Glands, 9.

Of these 7 were cervical glands and the other two glands in the Axilla. All except one advanced case are doing well.

Tuberculous Peritonitis, 4.

This condition responds well to radium treatment.
One case died 6 months after apparent recovery.

Uterine Hæmorrhages, 4.

Three results satisfactory. One only slightly improved.

Angiomata (cavernous), 14.

These cases give consistently good results. I find fractional doses are essential, especially in young children.

Lupus Vulgaris, 2.

One improved. One lost trace of.

Lupus Erythematosus, 2.

Results not very satisfactory so far.

Keloids, 3.

Results very satisfactory.

Dupytron, 6.

Three excellent results; two improved; one failure.

Summary of Malignant Cases treated during the last Seven Years, 1930–1936.

Carcinoma of the Ear.

			Alive after						
			1	2	3	4	5	6	7 years.
1930	3	...	2	2	1	1	1	1	1
1931	0	...	–	–	–	–	–	–	–
1932	0	...	–	–	–	–	–	–	–
1933	2	...	2	1	1	1	–	–	–
1934	4	...	3	3	3				
1935	4	...	3	2					
1936	5	...	3						

Carcinoma of Cheek.

			Alive after			
			1	2	3	4 years.
1933	4	...	4	3	3	2
1934	6	...	5	5	4	
1935	4	...	3	3		
1936	3	...	1			

Carcinoma of Tongue.

			Alive after						
			1	2	3	4	5	6	7 years.
1930	5	...	3	2	1	1	0	0	0
1931	4	...	2	1	1	1	0	0	
1932	8	...	4	3	3	2	1		
1933	3	...	2	1	1	0			
1934	5	...	2	2	1				
1935	9	...	5	2					
1936	10	...	3						

Carcinoma of the Lip.

			Alive after						
			1	2	3	4	5	6	7 years.
1930	5	...	3	2	2	1	1	1	1
1931	3	...	2	2	1	1	1	0	
1932	7	...	5	4	4	3	2		
1933	6	...	5	4	4	2			
1934	7	...	5	5	3				
1935	8	...	6	2					
1936	8	...	3						

Carcinoma of the Palate.

			Alive after 1	2	3	4	5	6	7 years.
1930	2	...	1	1	1	1	0	0	0
1931	2	...	1	1	1	1	0	0	
1932	4	...	3	2	0	0	0		
1933	2	...	2	2	2	2			
1934	1	...	1	1	0				
1935	2	...	1	1					
1936	1	...	–						

Carcinoma of the Tonsil.

			Alive after 1	2	3	4	5	6	7 years.
1930	1	...	1	0	0	0	0	0	0
1931	2	...	1	1	0	0	0	0	
1932	3	...	2	2	1	0	0		
1933	2	...	1	1	1	1			
1934	5	...	3	2	1				
1935	3	...	2	1					
1936	1	...	–						

Carcinoma of the Larynx and Pharynx.

			Alive after 1	2	3	4	5	6	7 years.
1930	3	...	1	0	0	0	0	0	0
1931	4	...	1	0	0	0	0	0	
1932	5	...	1	1	1	0	0		
1933	1	...	1	0	0	0			
1934	2	...	2	0	0				
1935	3	...	2	1					
1936	1	...	–						

Carcinoma of the Breast (Primary).

			Alive after 1	2	3	4	5	6	7 years.
1930	5	...	2	1	0	0	0	0	0
1931	4	...	2	1	1	1	0	0	
1932	0	...	–	–	–	–	–		
1933	2	...	1	1	0	0			
1934	2	...	1	1	1				
1935	5	...	3	1					
1936	4	...	1						

Carcinoma of the Breast (Secondaries).

			Alive after 1	2	3	4	5	6	7 years.
1930	6	...	3	0	0	0	0	0	0
1931	6	...	2	1	0	0	0	0	
1932	0	...	–	–	–	–	–		
1933	11	...	2	1	1	1	0		
1934	14	...	4	1	0				
1935	7	...	3	1					
1936	8	...	2						

Carcinoma of Cervix Uteri.

			Alive after 1	2	3	4	5	6	7 years.
1930	4	...	2	2	1	1	1	1	1
1931	3	...	1	1	1	1	1	0	
1932	8	...	5	4	4	3	1		
1933	3	...	2	2	1	1			
1934	3	...	3	2	2				
1935	5	...	4	2					
1936	7	...	2						

I include no statistics for Carcinoma of the Body of the Uterus, firstly because I do not often meet them, and secondly because I consider surgery offers greater hope of success.

Early in 1936 I procured a supply of radium in needles, and these I use in all Carcinomas of the Lip, Cheek, Tongue, and Buccal Cavity in general, and also for many cases of Epithelioma of the Skin Surfaces.

I still find it very difficult to induce patients to report regularly, and those cases which I have been unable to trace I consider to have died.

No. 49.

THE CHEMICAL CONSTITUENTS OF LICHENS FOUND IN IRELAND—*LECANORA SORDIDA* TH. FR.

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[Plate 16.]

[Read February 23. Published separately July 22, 1937.]

The first recorded study of the lichen *Lecanora sordida* would appear to be that made by Paterno in 1876 [Atti del. Re. Ac. d. Lincei (2), 3]. In the lichen Paterno found Usnic acid, Zeorin and Sordidin. To Zeorin Paterno attributed the formula $C_{13}H_{22}O$, while Hesse, who isolated it from *Placodium saxicolum* [J. Prak. Chem., 58, p. 480], gave it the formula $[C_{13}H_{22}O]_4$. To Sordidin has been given the formula $C_{13}H_{10}O_8$. Zopf isolated from *Lecanora sulphurea* [Ann. de Chem., 327, p. 322] Usnic acid, Zeorin, and Sordidin, and maintains that Paterno was not in reality dealing with *Lecanora sordida,* for in the latter Zopf found no Zeorin or Sordidin [Ann. de Chem., 295, p. 271].

In his examination of *Lecanora sordida* [Pers], Zopf [Ann. der Chem., 295, p. 268] found besides Atranorin a new acid, Zeoric acid. Hesse, in his examination of the same lichen [J. Prak. Chem., 58, p. 488], found Atranorin and Parellic acid. Hesse characterises Zopf's Zeoric acid as impure Parellic acid, and founds his opinion on the discovery that, when the acid is isolated according to Zopf's method, it has properties corresponding to those described by Zopf. Zopf retorts to this [Ann. der Chem., 327, p. 345] with an analysis of Zeoric acid, and shows that the latter has a higher hydrogen content than Parellic acid.

Hesse also investigated a second variety of *Lecanora sordida* [var. Schwartzii] [J. Prak. Chem., 58, p. 88], and isolated from it Atranorin, Thiophanic acid $[C_{12}H_6O_{12} + H_2O]$, Lecasteride $[C_{10}H_{18}O_3]$, Lecasteric acid $[C_{10}H_{20}O_4]$, and Roccellic acid $[C_{17}H_{32}O_4]$. The formulae given are those of Hesse, and the lichen was collected on the vine slope walls at Feuerbach. Zopf, in an investigation of what appears to be the same lichen [Ann. der Chem., 327, p. 342] collected in Arlberg, isolated Atranorin, Roccellic acid, and Thiophanic acid, and states that the Roccellic acid constituent is much the predominant, being present to the extent of 3 per cent.: he found no Lecasteric acid or Lecasteride present. Zopf further states that he was interested to investigate a greyer variety collected at Leschede, in Westphalia. This latter gave Atranorin [1·3 per cent.] and Roccellic acid [0·9 per cent.], but not a trace of Thiophanic acid. Instead of the latter there was present a colourless

acid, which crystallised from alcohol in prisms resembling stereocaulic acid, and dissolved in alkali with an intense yellow colour.

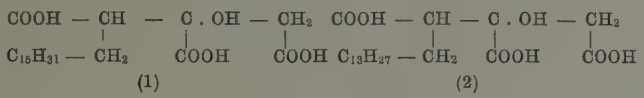
Leaving aside Paterno's work, regarding which the weight of evidence would appear to support Zopf's view, that Paterno had not really in hand a variety of *Lecanora sordida,* the conclusions may be drawn from the Hesse-Zopf results that the constituents of the lichen vary to a greater or lesser degree with the type, and possibly with the location. The results of the present investigation of the Irish lichen bear out Zopf's results with the lichen collected in Arlberg. Roccellic acid was present in predominant amount (4·1 per cent.), Atranorin and Chloratranorin (0·7 per cent.), Mannitol, and a very small amount of a body which is different in some respects from the body which Zopf identifies as Thiophanic acid, identical with Hesse's compound of that name. Whether the substance we have isolated from the Irish lichen (Fig. 1, Pl. 16) is really Thiophanic acid is by no means certain, for, though it has many of the recorded properties of Thiophanic acid, including the acidity and characteristic ferric chloride reaction of the latter, it differs from the recorded properties in the following respects:—It is a cream-coloured product, and not sulphur yellow, as described by Hesse; it melts at 258–260° C. instead of 242° C., as given in the literature; it crystallises from alcohol without any water of crystallisation, while Hesse attributes to it one molecule of water of crystallisation; its analysis differs fundamentally from that given by Hesse. We were led to suspect the accuracy of Hesse's analysis by the peculiar reaction of his Thiophanic acid with hydriodic acid. According to Hesse, under Zeisel conditions Thiophanic acid gives Thiophaninic acid, no evidence of methoxyl is obtained, but *iodine is liberated in the course of the reaction.* This latter phenomenon indicated the possible presence of chlorine in the molecule, and in fact the substance obtained by us contains 14 per cent. chlorine. To assure ourselves of the accuracy of our data for this chlorine-containing product two separate investigations were carried out—one on the lichen collected at Howth, North Dublin, and another on the lichen collected at Rosslare, Co. Wexford. The results were in complete accord, as the following figures show:—

		C.	H.	Cl.
North Dublin Lichen	...	55·03	3·91	14·03
Wexford Lichen	...	55·1	3·73	13·9
Hesse's data	...	42·66	2·08	—

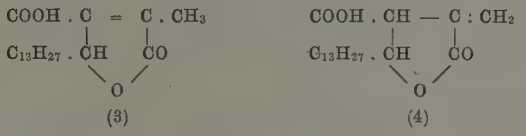
For the product, the formula $C_{24}H_{20}O_9Cl_2$ [C = 55·1; H = 3·82; Cl = 13·6] is suggested. The small amount of the substance available did not permit of any investigations of structure.

As regards the constitution of Roccellic acid, we confirm Hesse's formula, $C_{17}H_{32}O_4$. Hesse's statement that it is a dibasic acid has been confirmed by the preparation of the dimethyl ester: in addition, we find the acid to be dextrorotatory. The ease with which it forms an anhydride suggests the probability

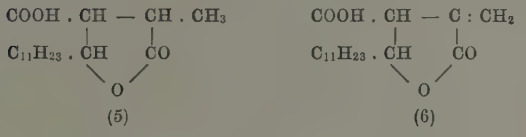
of its being a substituted succinic acid, though acids of the substituted glutaric type and higher homologues are not excluded. In considering possible constitutions attention may be directed to the structural configurations of certain fatty acids already isolated from lichens. The naturally occurring fatty acids, (1) Agaricic acid, (2) Caperatic acid, (3) Lichesteric acid, (4) Protolichesteric acid, (5) Nephrosteranic acid, and (6) Nephrosterinic acid (Asano Ber., 66, p. 1020. Ber., 68, p. 991. Asahina Ber., 70, p. 227)



(1) (2)



(3) (4)



(5) (6)

have as a common feature a structure of the substituted succinic acid type. In the course of his experiments Asano (*loc. cit.*) obtained the following:—

α methyl α′ tetradecyl succinic acid M.P. = 134°–136° C.
α methyl α′ hexadecyl succinic acid M.P. = 133°–136° C.

In view of the above it was decided to investigate Roccellic acid (M.P. 131°–132° C.) on the basis of its being α methyl α′ dodecyl succinic acid. Three methods of attack were employed in the degradation experiments—(*a*) Electrolytic decomposition by Walker's method, with the production of an unsaturated hydrocarbon, and subsequent fission by oxidation of this latter. While a hydrocarbon was obtained in the electrolysis, no satisfactory amount of fatty acid could be obtained from it by permanganate oxidation. (*b*) Application of the Wieland reaction, namely, interaction of the ester with phenyl magnesium bromide to form a di-tertiary alcohol, dehydration of the latter to an unsaturated hydrocarbon, and fission of this last by oxidation to benzophenone and the fatty acids. This method failed at the first stage, as an intractable gum was formed. (*c*) The production of a substituted bromine derivative by the Hell-Volhard Zelinsky method, dehalogenation of the resulting compound with production of a double bond, and subsequent fission by oxidation. Auwers, in an extensive research on the determination of the structure of aliphatic acids by means of bromination (Ber., 24, p. 2209, p. 2233), shows that all aliphatic carbonic acids with an α hydrogen atom can be

brominated, and that monocarbonic acids give practically only α monobrom compounds. In the case of dicarbonic acids in which the α hydrogen is found next each carboxyl group, dibrom compounds are formed readily. This rule has been confirmed for malonic, glutaric, methyl glutaric, adipic acids, etc. There are, however, exceptions, for succinic acid gives with difficulty s-dibrom succinic acid, the monobrom compound predominating. On the other hand, monomethyl succinic acid readily gives a dibrom compound, which shows a great tendency to lose hydrobromic acid. In the case of s-dimethyl succinic acid this tendency to lose hydrobromic acid is so enhanced that dimethyl maleic acid is the sole product. In the case of Roccellic acid, under the conditions described in the experimental portion, very little bromination took place; on the other hand, partial isomerisation occurred with the production of an acid of M.P. 81° C. The two isomers were separated by their different solubilities in chloroform.

With the failure of the degradation methods the problem was attacked from a synthetic end, and α methyl α' dodecyl succinic acid was prepared by the method used by Asano for the preparation of analogous substances. Two acids were isolated, M.P. 131° C. and M.P. 81° C., respectively. The former agreed in all particulars with natural Roccellic acid, and in addition the anils and p-nitro phenyl hydrazine derivatives were identical in each instance. The synthetic product of M.P. 81° C. was found to be identical with the acid obtained by isomerisation of the natural product, so that Roccellic acid may be accepted as α methyl α' dodecyl succinic acid. It is thus a reduction product of Nephrosterinic acid obtained by Asahina (*loc. cit.*) from the lichen *Nephromopsis endocrocea.*

Experimental.

Lecanora sordida is stated by Miss Knowles (The Lichens of Ireland, Proc. R.I.A., 38, Sec. B, No. 12) to be common on siliceous rocks around the coast, and also on mountains. We found it to be common at Howth and Killiney, and also along the Wexford coast, but in no case is it really abundant. It is found in patches of about 15 square inches, and the collection of it is tedious and difficult.

Ether Extract.

The lichen was collected in early September, 1934, from rocks on the east and south side of the Howth peninsula. 125 g. were ground, and covered with 700 c.c. ether, and left with occasional shaking for three days, at the end of which time it was filtered and the green filtrate distilled: a greenish residue was obtained. Subsequent extractions with 500 c.c. and 250 c.c. of ether removed all the material soluble in this solvent. The total extract weighed 8·25 g. It was treated with 50 c.c. ligroin, and the solution decanted from a small amount of gum. The ligroin solution was evaporated to dryness, and the residue warmed with 15 c.c. alcohol. The solution was decanted from a small

amount of solid (A), and to the solution was added 6 c.c. water, and the whole reheated: practically all went into solution. This solution on cooling deposited crystals (B). These were filtered, washed with 6 c.c. of 70 per cent. alcohol, and to the filtrate was added a large volume of water: this dilution threw down an oily product which on standing solidified (C).

The solid C when dissolved in alcohol did not give a greenish blue coloration with ferric chloride, but a colour somewhat more reddish violet than atranorin. It was dissolved in 3 c.c. alcohol, and the solution allowed to stand for two days: the crystals which had separated were collected and recrystallised from alcohol. Cream-coloured crystals were deposited, which in alcoholic solution gave a greenish blue coloration with ferric chloride. Recrystallisation was repeated until the mother liquors gave a greenish blue coloration with ferric chloride. Yield, 27 mg. M.P. 258°–260° C.

Analysis.

3·977 mg. gave 8·025 mg. CO_2; 1·400 mg. H_2O.
6·580 mg. gave 3·730 mg. AgCl; Methoxyl negative.

Found C = 55·03; H = 3·81; Cl = 14·03.

A sample isolated from the lichen collected at Wexford gave the following results on analysis:—

4·856 mg. gave 9·805 mg. CO_2; 1·640 mg. H_2O.
5·309 mg. gave 2·980 mg. AgCl.

Found C = 55·1; H = 3·73; Cl = 13·9.

$C_{24}H_{20}O_9Cl_2$ requires C = 55·1; H = 3·82; Cl = 13·6.

The product dissolves in strong sulphuric acid with a yellow coloration. It is soluble in potash to a yellow solution. It is extracted completely from ether by dilute sodium bicarbonate, and an alcoholic solution gives a weak acid reaction to moist litmus paper. The colour reactions with ferric chloride are as indicated above, and the substance does not lose weight when dried in high vacuum at 100° C.

The gum-like residue insoluble in ligroin and the product A were combined, boiled with 14 c.c. ethyl alcohol, cooled, and filtered. The residue thus obtained was crystallised from 11 c.c. benzol, and the crystals deposited on cooling filtered off. They had the characteristic structure of Atranorin, and an alcoholic solution gave with alcoholic ferric chloride a port-wine colour: M.P. 196–197° C.; weight 0·25 g.

Analysis.

4·677 mg. gave 10·135 mg. CO_2; 1·950 mg. H_2O.
3·511 mg. gave 2·170 mg. AgI.
13·720 mg. gave 1·750 mg. AgCl.

Found C = 59·1; H = 4·63; Cl = 3·15; OCH_3 = 8·15.

65 per cent. Atranorin/35 per cent. Chloratranorin requires C = 59·2; H = 4·58; Cl = 3·05; OCH_3 = 8·05.

The product B was recrystallised from 70 per cent. alcohol until the mother liquors gave no coloration with ferric chloride. The product thus obtained melted at 131° C. (Fig. 2, Pl. 16). Weight, 5·1 g.

Analysis.

4·728 mg. gave 11·760 mg. CO_2; 4·510 mg. H_2O.

Found C = 67·8; H = 10·6.

$C_{17}H_{32}O_4$ requires C =68·00; H = 10·66.

Specific Rotation.

0·63 g. in 25 c.c. alcohol in 20 cm. tube gave $\alpha = 0·88°$ $[\alpha]_D = 17·4°$.

Acetone Extract.

After extraction with ether the lichen was air-dried, covered with 500 c.c. acetone, allowed to stand for a week, and then boiled for two hours. On filtering and evaporating the acetone 2·65 g. of residue were obtained. It was partly crystalline, but had a red oil mixed with the crystals. The oil was removed by washing with 10 c.c. alcohol. The residue (0·83 g.) was crystallised from 25 c.c. benzene, and gave a product of M.P. 196°–197° C. (0·61 g.), identical with that of similar melting point obtained from the ether extract. No crystalline material was obtained from the oil soluble in alcohol.

Alcohol Extract.

After the acetone extraction the lichen was boiled for three hours with 500 c.c. alcohol. The alcohol on filtering and evaporating gave a wax-like solid. This was extracted with water, filtered from an insoluble residue, and the aqueous extract evaporated to dryness; this latter gave a semi-solid product, which was boiled with 30 c.c. alcohol and some Norite, and filtered. The hot filtrate on cooling deposited a small amount of gum-like material, which was discarded: the alcoholic solution was evaporated to one-third its bulk, when a small amount of white solid separated out. This on recrystallisation from alcohol gave a deposit of fine white crystals, M.P. 165° C., which were identified as mannitol.

Roccellic acid dimethyl ester.

1·3 g. of Roccellic acid were dissolved in 75 c.c. dry ether. 50 c.c. of an ethereal solution of diazo-methane were added, till a faint yellow colour remained in the solution. On standing overnight the ether was evaporated, and the product purified by slow crystallisation from ether. M.P. 28°–29° C.

Analysis.

3·697 mg. gave 5·230 mg. AgI.

Found OCH_3 = 18·7 per cent.

$C_{15}H_{30}(COOCH_3)_2$ requires OCH_3 = 18·9 per cent.

Anil of Roccellic acid.

0·3 g. of Roccellic acid was heated in an oil bath at 200° C. for three hours. 1·2 g. of aniline were then added, and the whole heated in a sealed tube for eight hours at 160°–170° C. The contents of the tube were then extracted with ether, the ethereal solution washed with hydrochloric acid and with water, and finally dried over sodium sulphate. The oily product obtained was spread on a porous plate, and then crystallised from a small amount of methyl alcohol. M.P. 57°–58° C.

Analysis.

3·265 mg. gave 0·124 c.c. Nitrogen at 30° C. and 748 mm.
Found N = 4·21.
$C_{23}H_{25}O_2N$ requires N = 3·92.

Interaction of Roccellic acid anhydride and p-nitrophenyl-hydrazine.

0·5 g. of Roccellic acid was heated at 160–170° C. for three hours, to form the anhydride: 0·3 g. of p-nitrophenyl-hydrazine was then added, and the whole heated in a sealed tube at 130–140° C. for five hours. The contents were then crystallised twice from 5 c.c. methyl alcohol (Norite), and the product obtained as light cream-coloured crystals. M.P. 113–114° C.

Analysis.

3·522 mg. gave 0·312 c.c. Nitrogen at 24° C. and 749 mm.
Found N =10·03.
$C_{23}H_{35}N_3O_4$ requires N = 10·07.

Attempted bromination of Roccellic acid.

1 g. of Roccellic acid and 0·1 g. of red phosphorus were placed in a modified Auwers tube and 1·5 c.c. of freshly distilled bromine added. The tube and contents were then heated at 100° C. for three hours. The contents were then removed, washed with warm water, and allowed to stand for a couple of days with a fresh supply of water. The white slightly oily solid thus obtained was first recrystallised from petroleum ether, and then warmed with a small amount of chloroform. The chloroform-insoluble fraction on thorough drying softened over a wide range, and finally melted at 108–109° C. It showed a positive bromine reaction.

Analysis.

5·215 mg. gave 12·960 mg. CO_2; 4·940 mg. H_2O.
8·146 mg. gave 0·150 mg. AgBr.
Found C = 67·42; H = 10·52; Br = 0·77.
$C_{17}H_{32}O_4$ requires C = 68·00; H = 10·66.

The fraction soluble in chloroform was recovered and recrystallised from ligroin. It melted at 81°–82° C. It gave a negative halogen test.

Analysis.

5·133 mg. gave 12·835 mg. CO_2; 4·94 mg. H_2O.

Found C = 68·18; H = 10·69.

$C_{12}H_{34}O_4$ requires C = 68·00; H = 10·66.

Synthesis of Roccellic acid.

A.—*Preparation of dodecyl iodide.*

90 g. of iodine and 6 g. of red phosphorus were placed in a round-bottomed flask. 18 c.c. of hydriodic acid (ρ 1·7) were gradually dropped into the flask, and the hydriodic acid gas thus produced after scrubbing through red phosphorus was passed into molten dodecyl alcohol. When the alcohol was saturated it was allowed to stand in a closed flask for two days, by which time a layer of strong aqueous hydriodic acid had separated at the bottom. The supernatant dodecyl dodecyl iodide was washed with water, taken up in ether, and dried over anhydrous sodium sulphate. The ether was distilled off and the residue distilled in vacuum, the portion coming over at 163–167° C. at 10 mm. mercury being collected. Yield 15·5 g. This method is much to be preferred to the interaction of phosphorus and iodine on the alcohol, the latter process giving considerable amounts of di-dodecyl phosphorous ester.

B.—*Preparation of propane $\alpha\alpha\beta$ tri-carballylic ester.*

This was prepared by the standard method, involving interaction of α brom propionic ester and sodium diethyl malonate. The fraction distilling at 176° C. at 30 mm. mercury was collected.

C.—*Condensation of propane $\alpha\alpha\beta$ tri-carballylic ester and dodecyl iodide.*

5·7 g. of the ester were placed in a small flask and 0·5 g. of sodium in 10 c.c. absolute alcohol added. The whole was shaken for 10 minutes, and then 6·5 g. of dodecyl iodide added, and shaking resumed for a short time. The contents of the flask were then transferred to a tube, which was sealed and heated at 130° C. for eight hours. The alcohol was then distilled off, the residue extracted with 75 c.c. ether, and from the ether extract the propane α dodecyl $\alpha\alpha\beta$ tri-carballylic ester recovered as an oil.

D.—*Hydrolysis of α dodecyl $\alpha\alpha\beta$ tri-carballylic ester.*

(*a*) The ester obtained as above was hydrolysed with 100 c.c. 10 per cent. alcoholic potash for 2½ hours by warming on a steam bath. The alcohol was then distilled off, the residue dissolved in water, the tri-carballylic acid precipitated with hydrochloric acid, and finally taken up in ether. The ethereal

solution was dried over anhydrous sodium sulphate, and the acid recovered by distilling off the ether.

E.—*Conversion of α dodecyl ααβ tri-carballylic acid into α methyl α′ dodecyl succinic acid.*

The acid was next partially decarboxylated and converted into α methyl α′ dodecyl succinic anhydride by heating at 130°–140° C. for three hours in an oil bath. The anhydride so formed was hydrolysed with 40 c.c. of 10 per cent. ethyl alcoholic potash for an hour on a steam bath, the alcohol evaporated off, and the residue dissolved in water to a clear solution. This solution was precipitated with dilute hydrochloric acid, when the free acid was obtained as an oily solid. The whole was extracted with chloroform: three layers were formed, a bottom chloroform layer, an emulsion over this, and an aqueous layer on top; this last was discarded. The chloroform layer was run off, and on evaporation a waxy white solid was obtained. On crystallising twice from ligroin the substance melted at 80–81·5° C.

Analysis.

5·130 mg. gave 12·760 mg. CO_2; 4·890 mg. H_2O.

Found C = 67·83; H = 10·58.

0·1 g. is neutralised by 6·8 c.c. N/10 caustic soda.

Found Molecular Weight = 294.

$C_{17}H_{34}O_4$ requires C = 68·00; H = 10·66: Molecular Weight = 300.

A mixture of this substance and the product of M.P. 81°–82° C. obtained by the isomerisation of Roccellic acid melted at 81·5° C.

The chloroform emulsion was filtered through a fluted paper, and the residue on the paper dissolved in 14 c.c. alcohol, filtered, and water added to the hot solution till a turbidity was obtained. On standing crystals were deposited. These were dissolved in 40 c.c. ligroin, filtered, and the ligroin solution on cooling deposited a white crystalline product, M.P. 131–132·5° C. (Fig. 3, Pl. 16). A mixture with natural Roccellic acid showed no depression in melting point.

Analysis.

4·606 mg. gave 11·525 mg. CO_2 and 4·430 mg. H_2O.

0·1 g. neutralised by 6·8 c.c. N/10 caustic soda.

Found C = 68·24; H = 10·68. Molecular Weight = 295.

$C_{17}H_{34}O_4$ requires C = 68·00; H = 10·66. Molecular Weight = 300.

The anil of this product was prepared in the same manner as that of Roccellic acid (*vide supra*). A product was obtained, M.P. 56° C., which showed no depression when mixed with the anil of the natural acid.

Analysis.

2·691 mg. gave 0·102 c.c. Nitrogen at 30° C. and 748 mm.

Found N = 4·20.

$C_{23}H_{35}O_2N$ requires N = 3·92.

Furthermore, a p-nitro phenylhydrazine derivative was prepared, as in the case of the natural acid. It melted at 113·5 C., and a mixed melting point with the derivative of the natural acid showed no depression.

Analysis.

2·958 mg. gave 0·263 c.c. Nitrogen at 749 mm. and 24° C.

Found N = 10·07.

$C_{23}H_{35}N_3O_4$ requires N = 10·07.

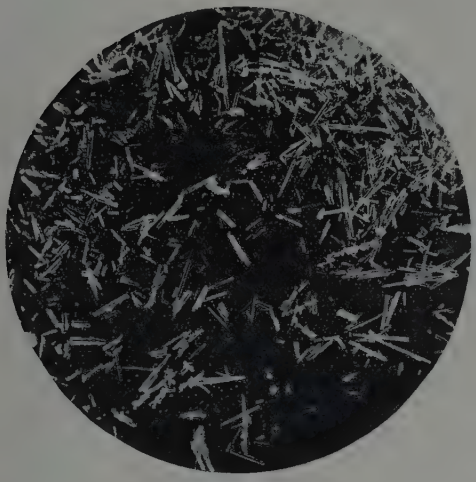


FIG. 1.

FIG. 2.

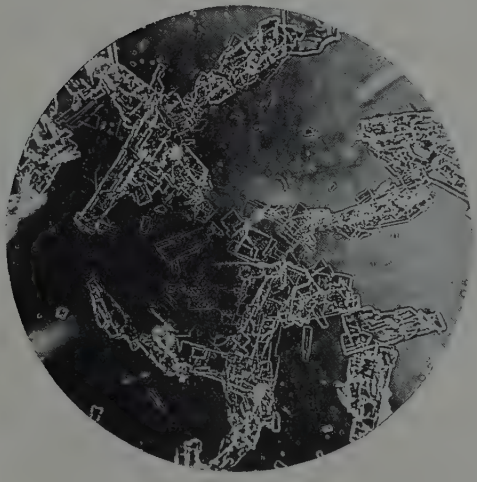


FIG. 3.

No. 50.

A TEN YEARS' EXPERIMENT ON THE SPREAD OF LEAF ROLL IN THE FIELD.

By PAUL A. MURPHY and J. B. LOUGHNANE.

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It is well known that leaf roll spreads in the field to healthy potato plants in the immediate vicinity, and that the distance to which it extends is comparatively small. Thus the healthy plants which are beside diseased ones are almost certain to become infected, those further removed are less certain, and so on until a point is reached beyond which the danger is slight. In an earlier experiment at Glasnevin it was found that the infections did not extend beyond three drills, and 88 per cent. of them occurred in the drill nearest the leaf roll sources, 8 per cent. in the second, 4 per cent. in the third, and none in the fourth drill.

This is typical, but the distance varies from year to year. It is of practical importance to determine the maximum and minimum spread over a period of years, and if possible the reason for the variation; and an experiment was begun in 1926 and continued on the same plan until 1935, in order to determine them under the conditions obtaining at Glasnevin. The plan of the experiment was as follows:—Nine leaf roll plants were grown in the form of a square in a plot of healthy President plants, as shown in Figs. 1 and 2, and double lines of the latter radiating to the N.S.E. and W. from the square were dug at the end of the season.

The two lines radiating to the east and west extended across five drills, or a distance of approximately 10½ ft. from the sources. The other two lines ran along the drills—that to the north extending to 7 plants, or a distance of 10½ ft., and that to the south to 14 plants, in order to minimize infection from outside, which was feared in that quarter. The danger proved to be practically non-existent, but most of the data were confined to the first seven plants. In other words, the amount of spread was determined each year on a total of 48 plants extending in the form of a cross with equal arms 10½ ft. long from the sources of infection. Actually, the spread was also determined to the 14th plant to the south, as shown in the last column of Table 1.

Each of these plants was dug separately, and its tubers were planted in a separate unit in the following year. If any secondary leaf roll developed in these in June it showed that the corresponding plant had contracted infection in the previous season.

The experiment was repeated year by year from 1926 to 1935 on the same plan, and in a similar open portion of the same field. The variety used

throughout was President, excepting the sources of infection, which were British Queen, as President is not suitable for this purpose owing to the small size of diseased plants and their liability to fail completely.

The general results are summarised in Table 1. It may be seen that the

TABLE 1.—*Spread of Leaf Roll,* 1926 *to* 1935.

Character of Year	Dates	Average per cent. leaf roll contracted by 48 plants within 10½ ft. of sources	Max. continuous spread extended to—		Furthest infected—	
			Plant	Drill	Plant	Drill
Bad years . . .	1926	75	7	4	14th	4th
	1929	70	9	3	14th	3rd
	1927	69	7	3	14th	5th
Intermediate years .	1930	57	3	3	7th	4th
	1932	[50]	5	—	12th	—
	1934	40	5	1	13th	3rd
	1933	25	1	2	7th	4th
Good years . . .	1928	15	3	0	3rd	3rd
	1935	12	3	1	3rd	1st
	1931	11	1	1	3rd	1st

years fall into three broad groups—the percentages of infection contracted within 10½ ft. of the diseased sources being 69 to 75 per cent. in the first, 25 to 50 per cent. in the second, and 11 to 15 per cent. in the third. It is concluded that the year 1930 properly belongs to the second group, the high percentage of disease being due in part to some leaf roll plants present in the supposed healthy stock, which spread infection before they could be removed. In another somewhat similar experiment carried out on uniform lines in 1928, 1929, and 1930, the percentages of infection with leaf roll were 15, 82, and 39, respectively, compared with 15, 70, and 57 in the present experiment, from which it may be concluded that the last figure is too high, and should probably be between 40 and 50 per cent. Part of the results for 1932 were lost owing to an accident.

In most years a continuous line of healthy plants nearest the sources contracted the infection, amounting to one to three plants in the best years (Fig. 1), three to five in the intermediate ones, and seven to nine plants in the bad years (Fig. 2). Averaging all the years, the maximum continuous spread

along the drills extended to 4·4 plants, or a distance of 79 inches. Similarly, the average spread extended across 2 drills, or a distance of 51 inches. This means that in an ordinary year at Glasnevin no plant separated from a source of disease by less than about 80 inches along the drills and 50 inches across them is likely to escape infection. The disease, both in this and other similar experiments, has always been found to spread further along the drills than across them, corresponding no doubt to the closer spacing of the plants in the former case, which would facilitate the movement of apterous aphids. It is also noticeable that the disease sometimes spreads more easily at the edge of a plot than in its interior.

Outside the area of continuous spread there was usually a considerable amount of scattered infection, extending on the average to the 9th plant to the south, or as far again as the distance of continuous spread. In the best years no disease at all was found beyond the 3rd plant, but in the worst ones it always reached the edge of the plot to the south, thus extending at least to the 14th plant, so that its full extent could not be determined. The extension towards the north must have exceeded 14 plants, seeing that, as is shown below, it always spreads more in that direction than to the south. Similarly, the extension generally exceeded the width of 5 drills in the worst years, thus reaching the edge of the plot.

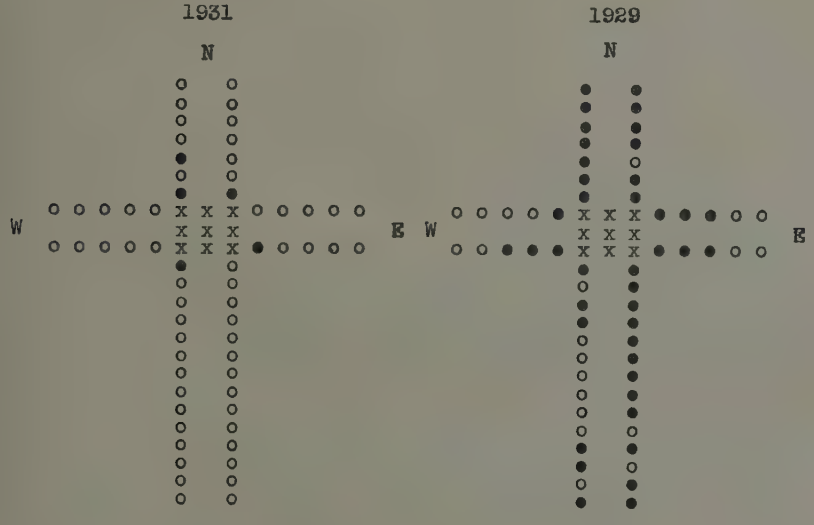

FIG 1. FIG 2.

In the intermediate years the scattered infections reached the 13th plant to the south and the 4th drill. Hence it may be concluded that in ordinary seasons at Glasnevin the chance of leaf roll infection in healthy stock is slight

if it is removed by a space of at least 14 plants or 5 drills, corresponding to 21 ft. and 10½ ft., from a source of disease, but in bad years this distance would not be sufficient.

The experiment permitted a quantitative determination of the amount of spread in four directions, and it is an interesting fact that on the average more infection occurred towards the north than the south, and more towards the east than the west. The results are shown in Table 2.

TABLE 2.—*Amount of leaf roll spread to N., S., E., and W., within* 10½ *ft. of sources of infection.*

——	Average per cent. leaf roll contracted within 10½ feet of source	Average continuous spread	Average distance of furthest infected plant
		Extent in inches	Extent in inches
Northward along drills . .	58	68	97
Southward „ „ . .	39	56	79
Eastward across drills . .	36	41	71
Westward „ „ . .	30	38	51

It will be observed that the three columns of the table are in agreement. The greater amount of infection to the north than to the south is shown in two ways—by the fact that the disease not only spread further, but also spread more freely, so that at any given distance from the sources there was regularly a higher percentage of leaf roll present. Similarly, there was a somewhat greater spread to the east than to the west. These facts are clear from Fig. 3, in which the percentages of the disease at gradually increasing distances from the sources are shown by smoothed curves.

It is most reasonable to attribute this somewhat uneven distribution to the prevailing south-west winds. Although Davies (3) has shown that winged aphids are not actually blown to potato fields, it is considered likely from these results that the prevailing winds must have an influence on the movement of the insects within the crop. It is concluded that most of the infection, owing to its strongly localized character, must be due to apterous forms, and it has been shown that these are in almost constant motion, and may cover a distance of 7 yards in 1 day under experimental conditions (1).

Prevalence of aphids in relation to the spread of leaf roll.—Notes were made of the presence of greenfly during each summer, but these were not on a quantitative basis at first, and were made by different observers, so that an exact comparison is impossible. Their prevalence, however, was outstanding in certain years, such as 1926 and especially 1929. The 1929 infestation was reckoned to exceed the bad infestation of 1921, and that of 1926 to equal it. These determinations were, of course, made before the amount of spread was known. The insects were not so prevalent in any subsequent year of the series.

The years 1926 and 1929 also stand at the head of the list for disease transmission.

In 1927, which was also marked by high transmission, the aphids reached the crop late, and developed slowly during June, but later became abundant in mid-July, and a very large amount of infection followed, the primary leaf roll which developed being unusually late. This was the one year in which, as will be seen, July was the most important month for leaf roll transmission. It may be remarked that the primary symptoms were most prevalent in the bad years of 1926, 1929, and 1927, but were rare in the good years of 1931, 1928, and 1935. When primary leaf roll did develop in the latter years it was sometimes conspicuous, so that its scarcity was not due to seasonal masking.

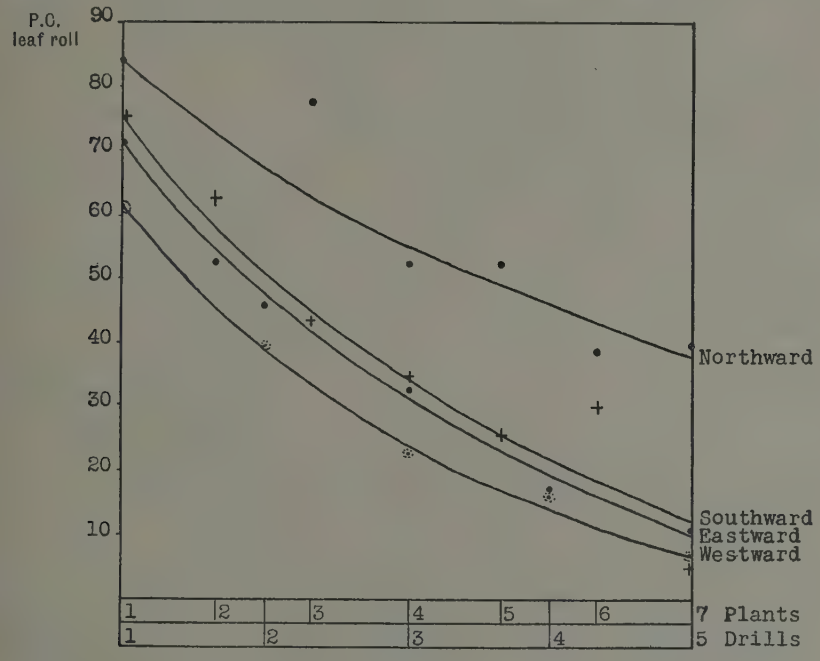

FIG 3.

At the other end of the series, the wet summer of 1931 was very notable for the absence of greenfly, and it also shows the smallest amount of leaf roll transmission, and incidentally of mosaic also. Aphids were not noted as particularly rare in 1928, and the small amount of spread of that year was connected with premature ripening of the plants during a hot dry period from July 9 to 25, following a wet June. It was noted that mosaic spread in that year more than leaf roll. There was no blight until August 24, which is unusually late for the district. Quantitative data were taken in most of the remaining years in connection with other experiments in the same field. These are summarised in Table 3.

In the earlier years the first greenfly were not observed until June 7 to 21, the usual date being June 10, when they were looked upon as new arrivals, whereas it is now known that the first arrivals begin from May 8 to 27, or very soon after the plants come up (May 1 or a little later), the usual date being May 15–16. These were alate *Myzus persicae* in every case without exception, *Macrosiphum gei* always coming later, and also leaving sooner. By about June 10, or 25 days later, the insects were numerous enough to be noticed on ordinary close inspection, and 45 days after the first arrival primary leaf roll appeared. This date varies with the time of arrival of the insects from June 20 to July 11, the usual date being June 29.

TABLE 3.—*Development of aphids on potatoes,* 1930 *to* 1935.

Year	Date infestation—				Maximum aphids per 100 leaves	Remarks
	Began	Rose to 25/100 leaves	Reached maximum	Fell to 25/100 leaves		
1930	May 14	—	July 10[1]	Aug. 4	126	Very rapid increase in late June.
1931	(late)	—	—	—	—	Late in arriving, and extremely scarce throughout season.
1932	May 27	June 20	July 13	Aug. 6	206	Very rapid increase in late June and early July.
1933	May 8	May 25	June 28	July 15	114	Unusually early arrival, development, and decline. Heavily parasitized.
1934	May 13	—	—	—	—	Abundant in late June. Development about normal.
1935	May 17	June 4	July 18	Aug. 17	185	Slow development after June set in.

It is unfortunate that the interesting wet year of 1931 was missed, though it is known that the aphids were unusually late in arriving, and remained negligible in numbers. Of the remaining years, 1930, 1932, and 1933 fall into the intermediate group, and they all show uniform and moderate infestations, reaching maxima of about 100 to 200 per 100 leaves usually before mid-July. The comparatively slight infestation and transmission of the dry season of 1933 must be attributed to the aphids being parasitized. It was noted that the infections were very scattered in that year (compare last four columns of Table 1).

Comparing 1935 with these years, it is by no means easy to see why it should show so much less disease transmission. The aphid infestation was

[1] Date of first enumeration. The maximum may have occurred somewhat earlier, and reached a higher figure.

actually greater than in 1933, but the increase was slower and the maximum much later; and it appears likely, as Davies first suggested (7, 2), that this is a material factor. July and August were also unusually dry and warm, a fact which further restricted the spread, as discussed later.

There is an evident connection between the prevalence of aphids and the spread of leaf roll in the outstandingly good and bad years, and it is probable that the two are in direct proportion throughout, although the records are not uniform enough to establish this, especially for the years of moderate spread. It is probable that the aphid population was conditioned mainly by the weather, because the other known factors, such as position of the plot in relation to the winter hosts of the insects, were believed to be uniform. Table 4 shows a summary of the weather conditions for the 10-year period.

The factors affecting the aphid population may be considered under three heads—(1) those affecting its numbers in the previous autumn or spring, or what may be called the initial prevalence; (2) those affecting its rate of increase from May onwards within the potato crop; and (3) the time of arrival of the aphids on the potatoes. So far as initial prevalence is concerned, there is believed to be some evidence of a difference in the number of individuals reaching the potato crop in different years, and it would be reasonable to expect that such factors as the following might be important in this connection: the weather of the previous autumn, as determining the prevalence of over-wintering forms; winter weather, as determining the amount of survival; or spring weather, as determining the rate of reproduction before migration. There is no indication, however, of any constant difference between the good and the bad years in these respects, either in rainfall or mean temperature, whether determined month by month or during any combination of autumn, winter, or spring months, up to and including May (see Table 4).

The explanation may be that the conditions determining the initial prevalence in early May are so complex that they cannot be deduced from a simple weather table alone, but involve also such factors as the number and condition of the insects in the previous autumn, and the number of parasites and predators. It is reasonable to expect that these factors must have some importance, but it may be, on the other hand, that the usual variations in the initial prevalence are of minor importance. Little quantitative information is yet available on this point.

So far as the rate of increase of aphids on the potato crop is concerned, the best years were marked by excessively wet Junes, and it appears that the most important effect of the weather was in deciding the rate of multiplication of the insects during that month. As shown in Table 4, the figures for the June rainfall of 1928, 1935, and 1931, when there was least spread, were 233, 143, and 210 per cent. of the normal, the average being 195. The highest corresponding figure for any other June was 124, and the average for the three years of maximum spread was 99, and for the four intermediate years 67 per cent. It is easy to understand that a wet June would reduce aphid

TABLE 4.

Rainfall and Temperature during the period 1926–1935.

	RAINFALL mm.													MEAN TEMPERATURE °F.								
	PREVIOUS YEAR			CURRENT YEAR										PREVIOUS YEAR			CURRENT YEAR					
	Whole year	Second half	Last quarter	Whole year	First half	First quarter	Second quarter	March	April	May	June	July	August	Whole year	Second half	Last quarter	Whole year	First half	First quarter	April	May	June	July	August
1926	745	353	200	701	340	174	166	22	43	66	57	62	56	48·2	51·7	46·1	49·3	47·1	43·8	47·3	48·9	55·3	61·3	60·9
1929	791	388	229	709	190	93	97	7	31	34	32	56	124	48·7	51·1	45·6	48·4	45·0	40·3	44·8	49·2	55·1	58·9	58·9
1927	701	361	206	834	300	185	115	82	31	21	63	85	153	49·3	51·6	43·6	48·7	46·2	42·5	46·9	50·5	52·4	59·7	59·3
1930	709	339	330	976	376	242	134	107	29	58	47	77	131	48·4	51·8	45·4	48·2	45·3	39·6	45·5	50·7	56·7	58·7	57·9
1932	855	476	159	679	262	101	161	37	61	89	11	119	40	48·7	51·3	46·2	49·1	46·1	42·2	43·9	49·9	56·6	60·8	60·3
1934	528	235	147	702	259	120	139	70	59	41	39	46	81	49·9	52·7	44·5	50·4	47·1	42·0	45·7	51·9	59·3	63·0	58·7
1933	679	417	198	528	293	173	120	47	22	58	40	48	18	49·1	52·1	45·7	49·9	47·1	41·1	49·0	52·7	57·8	63·1	62·1
1928	634	534	209	791	403	236	167	107	12	36	119	37	101	48·7	51·1	44·8	48·7	46·3	43·1	44·9	49·7	53·8	59·1	57·1
1935	702	443	238	682	319	151	168	17	50	45	73	21	58	50·4	53·7	47·8	49·3	47·2	43·4	45·5	50·0	57·7	60·9	60·9
1931	976	600	159	855	379	106	273	37	75	91	107	94	87	48·2	51·2	45·2	48·7	46·2	40·5	46·5	51·3	57·7	58·9	57·1
Normals	—	401	203	710	309	157	152	50	48	53	51	66	82	—	—	—	—	—	—	45·5	51·1	55·8	59·4	58·7

multiplication on the potatoes, but two of the wet Junes (in 1928 and 1935) were followed by very dry Julys, which, it might be suggested, would be very favourable for the insects. Actually, however, this was not the case, as observation at the time showed, for the premature partial ripening which rapidly ensued proved a further unfavourable factor for the insects, rather than a favourable one. In the remaining good year (1931), the wet June was followed by an almost equally wet July, and had been preceded by a wet May, the rainfall for the 3 months being 172 per cent. of the normal, a figure which sufficiently accounts for the remarkable scarcity of aphids in that year.

There is no evidence that the rainfall of any other month was more than a subsidiary factor. The most important months would be in July and May, but no rule concerning them is evident, apart from what has just been said, and there were dry and wet examples in both good and bad years. The very wet July of 1932 did not prevent a 50 per cent. spread of the disease, and the dry July of 1934 was associated with an almost identical amount. The same is true of August and of May.

These considerations emphasise the importance of the June rainfall for the spread of leaf roll. Granted a normal initial infestation, it appears that the years of greatest spread, such as 1926, 1929, and 1927, may be marked by a slight superfluity or even a considerable deficiency during that month, so long as the plants are making good growth. In the typical case the temperature is normal, and if it is low the effect is to depress both growth and aphid population, leading to a delayed maximum if conditions are subsequently favourable, as in July, 1927. Both the growth of the plants and the infestation during that month were typical of the normal June. The low temperature of June, 1928, also retarded growth and insect development, as the late appearance of primary leaf roll showed, but the subsequent drought from July 9 to 25 rapidly ripened the plants and proved unfavourable for infection.

The contrast between these Junes and those of the years of least spread is clear enough, as already pointed out. The latter are marked by wet Junes, and either very dry Julys, which further retard the aphid increase by hardening the plants, or wet ones, which retard it more directly. The temperature is not far from normal, but may be as much as 2° F. above or below it.

Considering the intermediate group of years, it will be seen that they are all similar in having a low June rainfall, and a temperature above the normal during the same month. The conditions during May and July were more variable. These years included the hot and dry Junes and Julys of 1933 and 1934, and the exceptionally dry June of 1932. It would probably be concluded at first glance that these, if any, were the proper conditions for maximum aphid infestation and infection, but observation showed that this was not the case.

The most reasonable explanation of the association of such conditions with moderate disease transmission is probably to be found in the different effects exercised on aphids, parasites, and plants. The aphids were heavily parasitized in 1933, which led to a reduced maximum, and an unusually early disappear-

ance of the insects. It is well known that rapidly-growing young plants are easiest to infect (5), and any direct effects of the weather in favour of the insects would probably be counterbalanced by the hardening and prematuring effect on the plants. This would be expected to make them less congenial to the insects, thus checking the numbers of the latter, and also certainly less easy to infect, with the result that they would pass out of the most susceptible stage more rapidly.

So far as the time of arrival of the aphids is concerned, this is of importance in relation to the state of development of the plants. If the crop is normally advanced, a late arrival generally means a delayed maximum and reduced infection. The year 1935 illustrates the working of these two factors. If both arrival and crop are late, heavy infection is still possible, as in 1927. A corollary of this is that the critical period is more likely to occur in July or later in northern districts.[2] The heavier infection recorded by Wellensiek (6) following late planting is probably to be explained principally on similar grounds. Granted a normal arrival and crop development, it appears that conditions which permit rapid growth of the young plants and rapid insect increase are most favourable for infection, for most transmission took place when the June rainfall and temperature were about normal or a little above. After June, the plants begin to pass out of the most susceptible period, and this is hastened by hot, dry Julys, which cause premature hardening or ripening.

The average time of arrival of the aphids at Glasnevin, May 15–16, is notably earlier than that given for Bangor, North Wales, at the other side of the Irish Sea, namely, early June (1, 2). In spite of this the infestation appears generally to have reached much higher figures in Wales, the figures for average total infestation for 1930, for example, being 887, compared with 126 at Glasnevin. The highest maximum for all aphids recorded at Glasnevin was 206 per 100 leaves in 1932, but a maximum of about 1,300 for *Myzus persicae* alone was recorded in the same year in Wales. These differences must depend mainly on the number of aphids reaching the potato crop in the two places.

Myzus persicae may reach potato foliage from at least two sources—the sprouts of the planted tubers (4, 1), or other plants, such as cabbages, on which it hibernates (2). In the first case a very early outbreak results, for the insects are present on the plants from the beginning. This did not happen in any of the present experiments, but the insects presumably originated from cabbages, which were present every year within about 200 yards of the potatoes, and on which *Myzus persicae* is perennial at Glasnevin. The proximity of such sources to potato fields has no doubt a material influence on the earliness of the infestation.

These considerations reduce themselves to the following general rule :—

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[2] This may be the case in Scotland according to recent work by Cockerham and McBain. (Rept. Director of Research, Scot. Soc. Res. Plant Breeding, 1936.)

Conditions which permit normally rapid growth of the young plants concomitantly with rapid increase of the aphids are most favourable for the spread of the disease. This presupposes that the aphids reach the crop at the normal time, that is very soon after the plants come up, the average date at Glasnevin being May 15. If the arrival is late and the crop normally advanced infection is somewhat reduced, as in 1932. If both the arrival and the development of the crop are late, July becomes the critical month instead of June, and heavy infection may follow, as in 1927. These various considerations point clearly to the importance of the month of June for the spread of leaf roll, or, put in more general terms, the period of six or seven weeks before flowering. This is a conclusion which is also deducible from experiments conducted under glass, and it is believed that the results can be applied to the field with slight modification, as has already been suggested (5).

As already pointed out, primary leaf roll begins very regularly at Glasnevin about June 29. The incubation period in young plants of that age (22 days after planting under glass, corresponding to about 52 days in the field) averages 37 days, indicating infection about May 23. As the plants get older (60 days after planting under glass, corresponding to about 90 days in the field) the incubation period lengthens considerably, with the result that infections contracted about July 1 or a little later take 40 to 60 days to show up, and would, therefore, be expected to appear from August 10 to 30. After this no more primary leaf roll develops. Some later infections follow, which do not manifest themselves until the following year, but it may be concluded that most of the disease is contracted from the last week in May until the first week in July, a period of about six weeks. The period during which the primary symptoms develop is much longer, and covers about nine weeks.

The weather of the three summers of maximum spread was not abnormal, and examination of the meteorological records from 1901 to 1925 shows that 7 years out of the 25 had comparable Junes. A corresponding spread of leaf roll would be expected in all those years at Glasnevin and other places with similar weather conditions. Even in the drier parts of Ireland no such general extension of the disease takes place, outside of gardens and urban areas, and the limiting factor, therefore, cannot be climatic, but must consist of the number of sources of infection and the prevalence of *Myzus persicae*. Of these, the latter is of primary importance, because the sources will not be long absent where it is regularly abundant. It is necessary not only for the insects to be present, but to be present early in the season in potato fields; and this no doubt is the real limiting factor. This means, in practice, that, outside of places where June is regularly wet, the health of the potato crop depends on the scarcity of the winter host plants of *Myzus persicae* and their remoteness from potato crops.

The correctness of this reasoning is shown by the ready spread of leaf roll and mosaic in urbanized areas in eastern and central Ireland, and its absence on open farms under the same climatic conditions. It also explains the

abundance of these diseases in industrial and thickly populated countries, and their endemic appearance in places which at one time grew healthy potatoes, like the Central Experimental Farm at Ottawa. The outbreak of the curl in eighteenth-century England was no doubt due to the importation of new viruses, but it was also a symptom of the growing industrialisation of the country.

Acknowledgments are due to Mr. R. McKay for general assistance during the earlier years of the work.

Summary.

In a field experiment conducted on uniform lines and under the same conditions for ten years the extent of the spread of leaf roll was determined in relation to weather and aphid population.

The years fell into three classes—the percentage of infection within 10½ ft. (3·2 m.) of the sources of disease being 75 per cent. or less in the first, 50 per cent. or less in the second, and 15 per cent. or less in the third.

The minimum spread of infection extended to the 3rd plant along the drill, and to the 1st to 3rd drill, and the maximum exceeded the 14th plant and 5th drill, and was not determined.

In an average year at Glasnevin no plant separated from the sources by less than 80 inches (2 m.) along the drills and 50 inches (1·3 m.) across them is likely to escape infection. Under the same conditions, the chances of infection are slight in plants removed more than 21 ft. (6·4 m.) along the drills and 10½ ft. (3·2 m.) across them from leaf roll sources.

The disease regularly spread somewhat more to the north than to the south, and slightly more to the east than to the west, due probably to the prevailing S.W. winds.

Aphids were notably most abundant in the years of greatest spread (1926 and 1929), least so in the year of minimum spread (1931, and to a less extent in 1935), and were probably in similar proportion throughout.

It is concluded that most of the leaf roll infection took place during 6 weeks from the end of May to early July. The resulting primary leaf roll appeared over about 9 weeks from the end of June to the end of August, the incubation period lengthening from about 37 days at first to 40 to 60 days later. In late seasons July or later becomes the principal infection period, and this is probably the rule in northern districts.

The three years of least spread had excessively wet Junes. The most important effect of the weather was in restricting aphid increase during the critical period, and this was reinforced by very dry Julys, which hardened the plants, or by a continuation of the wet weather.

The conditions producing maximum infection were comparatively normal rainfall and temperature in June, permitting rapid growth of the plants and simultaneous increase of the aphids.

The years of moderate infection were marked by low rainfall and high temperature in June, these conditions being favourable for the aphids (apart from parasites), but tending to ripen the plants prematurely.

Myzus persicae hibernated each year in an active condition on cabbages in a neighbouring garden, and no doubt reached the potatoes from this source.

The general absence of leaf roll from farms in eastern Ireland, where the conditions correspond to those at Glasnevin, must be due to the scarcity of the winter hosts of *Myzus persicae* near potato fields, seeing that weather conditions in June are so often favourable for maximum infection.

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No. 51.

INVESTIGATIONS ON A MOLECULAR CONSTANT FOR SOURED MILKS.

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[Communicated by Prof. T. J. Nolan.]

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In an earlier paper (1) the writers described a new and improved cryoscopic constant for milk based upon three fairly simple estimations, viz., the refractive index and chloride content of a modified Ackermann serum and the soluble phosphate content of the milk. The constant arrived at in this way reproduced the osmotic contribution of all the chief active constituents fairly accurately, and corresponded thus to an approximate analytical determination of the basic physiological constant of milk—its osmotic pressure, or what amounts to the same thing, its freezing-point depression. It was accordingly capable of use as a criterion for the detection of added water in milk, and examples of its use and value for this purpose were given in the same paper.

Mohler and Forster (2) have recently confirmed these findings in a comparative study of various methods for the detection of added water, and have also used this constant as the basis of a formula applicable to watered cream.

This and similar constants have, however, an important disadvantage in practice—they can only be employed with fresh samples of milk. As it frequently happens that considerable souring has occurred in the suspected sample before it can be analysed, the need for a method or a cryoscopic constant applicable also to soured samples is obvious. This point was referred to in the previous paper, and it was promised that it would receive attention in a later communication. The present paper is an account of some investigations on this aspect of the matter.

Effects of souring on Cryoscopic Constant. Modifications in type of serum, etc.

As the constant under discussion depends on three estimations, viz., refractive index, chloride, and soluble phosphate, the effect of souring on each must be considered separately.

(*a*) *Refractive Index.*—The method employed for the preparation of the previous serum used was that of Ackermann (3), replacing the precipitant calcium chloride by an equivalent concentration of calcium nitrate so as to permit of the determination of milk chloride in the same serum. Now many authors have shown (4), (5), (6), (7) that the refractive index of the usual milk sera, Ackermann's, Copper Sulphate serum, etc., rises during the early

stages of souring, and often falls later, and that this variation cannot readily be correlated with the degree of acidity developed. A serum recently described by Rothenfusser (8) which employs basic lead acetate as precipitant is relatively free from these defects however, and has in fact the remarkable property of remaining almost constant in refractive index for a given sample of milk, whether fresh or sour. It seemed that the use of this serum coupled with appropriate determinations of chlorides and phosphates might afford a solution of the sour milk question, and it has therefore been examined for this purpose.

(*b*) *Chloride* cannot as heretofore be determined in the new serum, as a progressive diminution was found to take place in the chloride content of this (Rothenfusser) serum as souring proceeded. It may, however, easily be determined directly on a sample of the milk by the Drost method already used. The end point of the titration is, however, not quite so sharp and satisfactory as in the serum. An alternative and better procedure, available as soon as souring has gone far enough to cause a separation of curd and whey, is to use for the chloride determination the serum obtained by directly centrifuging the sample. It is advisable to shake with about $\frac{1}{10}$th of its volume of carbon tetrachloride before centrifuging to obviate rise of fat. About 10 minutes at 3,000 r.p.m. gives a clear separation.

(*c*) Soluble phosphate presents more difficulty, as increasing amounts are formed from the insoluble phosphates as souring proceeds. The method of direct titrametric determination previously employed would therefore give much too high values once the sample was no longer fresh. Fortunately the ratio of soluble to total phosphate is fairly constant in fresh milk, and the initial soluble phosphates can therefore be obtained approximately from the total phosphate, a figure which is not seriously affected by souring. Lenstrup's results (9) give a ratio of 57 per cent. for the phosphates, and the present writers found an almost identical result (56 per cent.) as the average of 16 determinations (extreme variation 50 per cent. to 59 per cent.). Total inorganic phosphate can be simply estimated by a modification of the titrametric oxalate method (10) which will be outlined later. Here again, as for chloride, the centrifugal serum from clotted samples is available, as it contains all the inorganic phosphate of the milk, and is in general to be preferred.

The Cryoscopic Constant for fresh and soured milks.

According to Rothenfusser the lead acetate serum from fresh milk contains all the lactose, 99 per cent. of the chloride (reckoned as NaCl) of the milk, and practically none of the phosphates and citrates. The other constituents are acetates of alkalies (mainly) and of alkaline earths equivalent to *all* the acetic acid present as acetate in the precipitant, a small amount of lead, and some albumin, in addition to the usual non-protein nitrogen. With souring the composition naturally changes, lactose being replaced by lactic acid and later in part by other substances, and additional lead and albumin remaining in solution, but in such a way that the refractive index remains almost unchanged. The refractive index at any time is therefore essentially a function of the

original lactose and chloride contents of the serum, mainly the former, as the other constituents are either relatively constant or unimportant.

The following approximate equations therefore hold (cp. Ryan and Pyne (*loc. cit.*)) for a milk containing L grams of lactose, C grams of chloride (as NaCl), and P grams of soluble phosphate (as P_2O_5) per 100 grams of water—

$$R_s - R_w = 3{\cdot}48\ L + 4{\cdot}52\ C + K_1 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (a)$$

Freezing pt. depression, $\triangle = 0{\cdot}052\ L + 0{\cdot}615\ C + 0{\cdot}585\ P + K_2$ . . . . (*b*)

where K_1 and K_2 are constants (K_2 relatively small), R_s and R_w the refractive indices of Rothenfusser serum and of water, respectively, in degrees Zeiss ($R_w = 15{\cdot}0$ at 17·5° C.), and the numerical coefficients in the first equation are the augmentations of refractive index over that of water for solutions containing 1 g. per 100 c.c. water of hydrated lactose and sodium chloride, respectively (cp. Rothenfusser and Kotschopoulos, 11), and those in the second equation the corresponding freezing-point depressions. (The phosphate is taken for this purpose as a mixture of primary and secondary salts of *pH* 6·6.)

Eliminating L gives

$$66{\cdot}9\triangle = R_s - R_w + 36{\cdot}6\ C + 39{\cdot}1\ P + K_3 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

Or

$$K = (R_s - R_w) + 36{\cdot}6\ C + 39{\cdot}1\ P,$$

where K is the cryoscopic constant. In practice C is determined by titration of 10 c.c. milk with $N/10$ $AgNO_3$. The factor 36·6 therefore becomes

$$36{\cdot}6 \times \left(0{\cdot}00585 \times \frac{100}{10}\right) \times \frac{100}{100 - F/0{\cdot}93} = \frac{100}{100 - F/0{\cdot}93} \times 2{\cdot}2,$$

where F is the percentage fat in the milk and 0·93 its density. (Rothenfusser has shown that the volume of serum obtained from a fat-free milk is approximately equal to the volume of the milk used, so that a correction need be made for fat content only in the case of those estimations carried out directly on the milk to bring all determinations to a common basis.) The soluble phosphate which is determined in practice (as will be seen later) by the titration of the *total* inorganic milk phosphate in 10 c.c. milk from tertiary to secondary with $N/10$ acid (soluble phosphate is taken as 56 per cent. of the total). For this the factor 39·1 becomes

$$\left(39{\cdot}1 \times \frac{56}{100} \times 0{\cdot}0071 \times \frac{100}{10}\right) \times \frac{100}{100 - F/0{\cdot}93} = \frac{100}{100 - F/0{\cdot}93} \times 1{\cdot}6.$$

The working form of the equation is therefore

$$K = R_s - R_w + \left(\frac{93}{93 - F}\right)\left[2{\cdot}2\left(\text{c.c.}\ \frac{N}{10}\ AgNO_3\right) + 1{\cdot}6\left(\text{c.c.}\ \frac{N}{10}\ \text{acid}\right)\right] \quad \ldots\ldots \quad (2)$$

If, however, chloride and phosphate are determined in 10 c.c. of the centrifugally separated serum (or the sour milk filtrate) instead of in 10 c.c. of the milk, the correction for fat disappears, that for the volume of precipitated casein need not be taken into account as it is relatively small, and the formula reduces to—

$$K = R_s - R_w + 2{\cdot}2\left(\text{c.c.}\ \frac{N}{10}\ AgNO_3/10\ \text{c.c. serum}\right) + 1{\cdot}6\left(\text{c.c.}\ \frac{N}{10}\ \text{acid}/10\ \text{c.c. serum}\right)$$

. . . . (3).

This method of working has the advantage not only of securing sharper end points for the chloride and phosphate titrations, but also of rendering a fat estimation unnecessary.

Analytical Methods.

(*a*) *Serum.* The basic lead acetate used should contain 20 per cent. lead, and have a basicity of 1/3rd. This corresponds to about 8·0 per cent. acetic acid as acetate, a sp. gr. of 1·213, and a refractive index of about 91 Zeiss. For the preparation of the serum 5 c.c. of the acetate is added to 100 c.c. milk in a fairly large cylinder provided with a stopper, so as to form a layer below the milk. The cylinder is stoppered, inverted, and very vigorously shaken to ensure complete mixing, and then filtered. Filtrate, which should be clear greenish, is ready for immediate use. Refractive index is determined by Zeiss dipping refractometer at 17·5° C.

(*b*) *Chloride* is determined by the Drost direct titration method (12) described in previous article, either on the sample or on its serum or filtrate.

(*c*) *Total inorganic phosphate* is obtained by a modification of the oxalate titration method described by one of the writers (*loc. cit.*). In this 10 c.c. of milk or corresponding sour serum or filtrate is made definitely alkaline to phenolphthalein, an excess of calcium chloride then added, and the milk then carefully neutralised to phenolphthalein with dilute acid. An excess of neutral potassium oxalate is now run in and converts the previously formed $Ca_2P_2O_8$ into K_3PO_4. The latter can now be titrated with acid to the secondary salt using phenolphthalein, and this titration measures the total inorganic phosphate 1 c.c. $N/10$ acid = 0·0071 gr. P_2O_5.

Check of Molecular Constant.

The table gives the results of a series of determinations on individual milks which were allowed to sour naturally in closed vessels in the laboratory at ordinary temperatures.

The figures under K are reasonably constant, particularly for the first 3–4 weeks of souring. They show on the whole, however, a tendency to fall slightly in the early stages of souring, and to rise again and even exceed considerably the initial values towards the end of the period (4–6 weeks), particularly in the case of those samples which had undergone souring at the higher summer temperatures. This is due almost entirely to corresponding variations in the refractive index, as the values for chloride remain naturally unchanged, and those for phosphate show only a slight early upward tendency at times (probably due to hydrolysis of small amounts of phosphatic esters, cp. Graham and Kay (13)). On the whole the values obtained suggest that this new sour milk "constant" is not much, if at all, inferior to the writers' earlier molecular constant for fresh milk. Thus, excluding the values found for samples which had stood longer than 3 weeks at the high summer temperatures, the range for K is now seen to be 32·51 to 34·42 (average 33·48), compared

Sample No.	Date	Acidity c.c. N/10 NaoH/10 c c.	$R_s - R_w$	Cl as c.c. N/10 $AgNO_3$	Total P as c.c. N/10 acid	K
Skim	28/5/35	Fresh	24·20	2·75	2·02	33·48
	3/6/35	10·50	24·0	2·70	2·12	33·33
	4/7/35	17·40	25·5	2·60	2·00	34·42
(59 A)	23/4/36	1·65	24·15	2·88	2·01	34·08
	4/5/36	9·20	24·15	2·75	2·21	34·11
	12/5/36	10·40	23·82	2·63	2·20	33·51
	19/5/36	10·30	24·0	2·77	2·12	33·87
	28/5/36	11·40	24·80	2·68	2·18	34·56
(66)	23/4/36	2·33	25·55	1·83	2·38	33·74
	4/5/36	10·45	25·70	1·90	2·38	34·05
	12/5/36	–	25·40	2·0	2·38	33·98
	19/5/36	11·05	25·80	2·0	2·38	34·38
	28/5/36	11·60	26·35	1·9	2·48	34·81
(85 B)	23/4/36	1·90	25·35	2·3	2·07	34·06
	4/5/36	9·75	25·20	2·20	2·18	33·86
	12/5/36	–	25·70	2·20	2·18	34·36
	19/5/36	11·05	26·70	2·25	2·21	35·53
	28/5/36	13·40	28·20	2·20	2·28	37·02
138	23/4/36	2·00	23·75	2·80	2·16	33·75
	4/5/36	9·80	23·50	2·70	2·25	33·42
	12/5/36	–	23·77	2·65	2·25	33·57
	19/5/36	10·90	24·32	2·60	2·28	34·06
	28/5/36	11·80	25·10	2·75	2·28	35·18
M 5	6/12/36	–	23·95	1·95	2·55	32·65
	12/12/36	–	23·90	1·95	2·55	32·60
	21/12/36	–	24·05	2·05	2·55	32·98
	12/1/37	–	24·50	2·05	2·55	33·43
	19/2/37	–	24·20	2·05	2·55	33·13
128	6/12/36	–	25·00	2·25	2·43	34·19
	12/12/36	–	24·70	2·25	2·43	33·89
	21/12/36	–	24·75	2·30	2·43	34·06
	12/1/37	–	24·70	2·30	2·43	34·01
	19/2/37	–	23·50	2·30	2·45	32·81
131	6/12/36	–	24·70	2·15	2·30	33·45
	12/12/36	–	24·50	2·15	2·30	33·25
	21/12/36	–	24·40	2·25	2·30	33·37
	12/1/37	–	25·00	2·25	2·30	33·97
	19/2/37	–	24·20	2·25	2·30	33·40
37	25/1/37	–	23·95	2·55	2·20	33·44
	4/2/37	–	23·60	2·50	2·20	32·98
	15/2/37	–	23·65	2·50	2·40	33·36
	24/2/37	–	23·80	2·60	2·20	33·41
60	25/1/37	–	24·25	2·55	1·80	33·05
	4/2/37	–	24·15	2·48	1·80	32·72
	15/2/37	–	24·65	2·48	1·98	33·52
	24/2/37	–	24·20	2·60	1·80	33·35
78	25/1/37	–	24·20	2·40	1·95	32·94
	4/2/37	–	24·00	2·30	1·95	32·51
	15/2/37	–	24·10	2·30	2·28	33·16
	24/2/37	–	24·00	2·20	2·23	32·58

with a range of 32·32 to 34·33 (average 33·14) for the fresh milk constant (*loc. cit.*). Undoubtedly the number of independent milk samples examined is not so great for the "sour milk" constant as for the other, and further work may therefore be not unlikely to widen the range of values somewhat, but probably not very greatly, for the theoretical basis of both constants in their application to fresh milk samples is approximately the same, and the maximum variation in the value of the "sour" constant during the period of souring of any individual sample (excluding those from the older high temperature samples) has in no case exceeded one unit. Incidentally, the values for the present sour milk constant lie a little higher than those for the previously described fresh milk one, in spite of the fact that the Rothenfusser serum used here and the Ackermann serum for the other have approximately the same refractive index. The explanation is undoubtedly the somewhat greater phosphatic correction added to the former, and made necessary by the fact that this serum, unlike that of Ackermann, is practically devoid of phosphates.

It is hoped to be able on another occasion to furnish evidence on further points of interest, e.g., on how far the influence of suitable storage temperatures and of preservatives may enable the question of adulteration in still older samples to be dealt with by use of these cryoscopic constants.

Summary.

In continuation of previous work on an improved cryoscopic constant for milk, a constant has been developed which is applicable to either fresh or sour samples. The analytical determinations required for its evaluation are three—viz., the refractive index of the Rothenfusser basic lead acetate serum, and the chloride and total inorganic phosphate contents of the milk or its centrifugal serum. The results of present experiments suggest that the degree of constancy attained is not much inferior to that of the earlier fresh milk molecular constant.

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No. 52.

THE CHEMICAL CONSTITUENTS OF LICHENS FOUND IN IRELAND—*PERTUSARIA CONCRETA* NYL. FORM *WESTRINGII* NYL.

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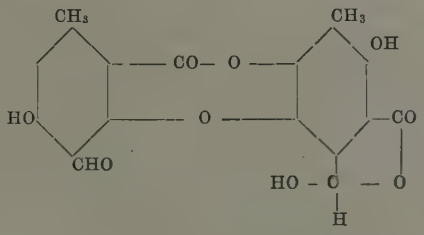
(Plate 17.)

[Read May 25. Published separately July 30, 1937.]

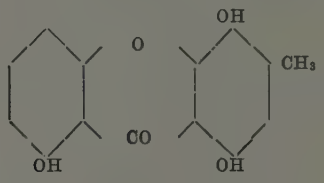
The lichen *Pertusaria concreta* Nyl. form *Westringii* Nyl. is stated by Knowles (Proc. R.I.A., **38**, B, p. 298) to occur around the coast of Ireland but to be comparatively rare. The sample investigated in the present instance was collected at St. Helen's, Rosslare, Co. Wexford, where it occurred plentifully in a restricted area. It was identified by Dr. P. O'Connor, of the Natural History section of the National Museum, to whom we wish to express our thanks.

The principal constituent of the lichen has been identified by us as norstictic acid $C_{18}H_{12}O_9$.



Two other substances occur in it in minor amount, mannitol and a chlorine-containing substance to which we assign the formula $C_{14}H_7O_5Cl_3$; this latter we call *Concretin*. Concretin crystallises from alcohol with three molecules of water of crystallisation which are readily lost in a high vacuum at 100° C.; its reactions strongly resemble those of that class of substances which is characterised by Zopf (Die Flechtenstoffe, p. 120) as the Thiophanic acid group. It appears, however, to be different from any known member of that group. It contains three hydroxyl groups, which are readily acetylated, and the acetylated product is insoluble in alkali. It appears to be difficult to methylate

completely with diazomethane. Whether the substance is a substituted methyl xanthone is doubtful. It is not a derivative of Ravenelin $C_{14}H_{10}O_5$



(Raistrick, Robinson and White, Biochem. J., **30**, p. 1303), for the trichlor-derivative of the latter, which we have prepared, gives a solution much deeper in colour than Concretin, gives quite a different coloration with sulphuric acid, and is slightly different in its reaction towards alcoholic ferric chloride. Unlike the case of Erdin (Raistrick and Smith, Biochem. J., **30**, p. 1315) the product obtained from Concretin on treatment with diazomethane contains no nitrogen. Owing to the very small amount of the substance present in the lichen it was not possible to proceed further with a determination of constitution.

Experimental.

Ether Extract of Lichen.

980 g. of air-dried lichen was allowed to stand with 3 litres of ether for 3 days, the whole being occasionally shaken; the ethereal solution was then filtered off. The extraction was repeated a second time, and the combined solutions evaporated to about 25 c.c.; during the concentration a white solid separated out; this was filtered off, and when dry weighed 0·7 g. It was crystallised from cyclohexanone, giving 0·5 g. of material which was found to be identical with the main acetone extract, and was nor-stictic acid.

To the ethereal mother liquors as obtained above was added 10 c.c. chloroform. On standing, a brownish solid was deposited; this was filtered off and crystallised from 3 c.c. methyl alcohol (Norite). The product was thus obtained as light sulphur-coloured needles, Fig. 1, Pl. 17. Weight, 37 mg.

Second and third groups of crystals were obtained as follows:—The ether, chloroform, and methyl alcohol mother liquors were combined, and to the whole 20 c.c. chloroform added. On standing, a solid was deposited which on recrystallisation from a small amount of methyl alcohol gave 12 mg. of pure product. The mother liquors from this product were added to the main mother liquors, and the whole allowed to concentrate by exposure to the air. The solid deposited was crystallised first from acetic acid and then from methyl alcohol, 18 mg. being eventually obtained. The total yield was thus 67 mg. The name Concretin is proposed for this material.

Analysis.

6·492 mg. when dried in high vacuum at 100° C. lost 0·832 mg.
4·838 mg. when dried in high vacuum at 100° C. lost 0·621 mg.
4·217 mg. (vac. dried) gave 7·215 mg. CO_2 and 0·780 mg. H_2O.
5·660 mg. (vac. dried) gave 6·740 mg. AgCl.
0·227 mg. (air dried) in 4·093 mg. camphor, 5·2° △. Mol. Wt. = 428.
0·219 mg. (air dried) in 3·592 mg. camphor, 5·4° △. Mol. Wt. = 452.
Found C = 46·66; H = 2·05; Cl = 29·45; $H_2O = \begin{cases} 12·8 \\ 12·9 \end{cases}$
$C_{14}H_7O_5Cl_3$ requires C = 46·47; H = 1·94; Cl = 29·4.
$C_{14}H_7O_5Cl_3 . 3H_2O$ requires M.W. = 415·5; H_2O = 12·99.

A methoxyl determination gave a negative result. Concretin melts with decomposition at 287° C.: it is readily soluble in ether: soluble with difficulty in chloroform: soluble in warm methyl alcohol and warm acetic acid. It is soluble in dilute sodium carbonate and sodium bicarbonate to form a yellow solution: soluble in concentrated sulphuric acid to a yellow solution which on heating is not decomposed but changes to a reddish yellow: on cooling this solution exhibits a strong green fluorescence. The substance is insoluble in concentrated hydrochloric acid. The coloration of an alcoholic solution with alcoholic ferric chloride was compared with the following substances:—

(1) Chlorine product from *Lecanora sordida*—greenish blue.
(2) Erdin—grass green.
(3) Trichlor ravenelin—greenish brown.
(4) Ravenelin—brownish green.
(5) Concretin—olive green.

Triacetyl-concretin.

20 mg. of concretin was suspended in 2·5 c.c. acetic anhydride, and a drop of concentrated sulphuric acid added: the whole went into solution, and on standing overnight a white solid had separated. The whole was diluted with water, the deposit filtered off, dried, and crystallised from 1 c.c. acetic acid. It was obtained in the form of clusters of needles, Fig. 2, Pl. 17. Yield, 15 mg. M.P. 220°–222° C.

Analysis.

For analysis the material was dried in high vacuum at 100° C.
4·908 mg. gave 8·820 mg. CO_2 and 1·250 mg. H_2O.
Found C = 49·01; H = 2·83.
$C_{14}H_4O_5Cl_3(CH_3CO)_3$ requires C = 49·23; H = 2·66.

Triacetyl concretin is insoluble in dilute sodium bicarbonate and sodium hydroxide. An alcoholic solution with alcoholic ferric chloride gives no colour reaction.

Methylation of Concretin.

20 mg. of concretin was dissolved in 10 c.c. ether and treated with diazomethane solution (1 c.c. nitroso methyl urethane): a yellowish precipitate was immediately formed. On standing for two hours the solvent was allowed to evaporate, the residue dissolved in 10 c.c. acetone, and the solution re-treated as before with diazomethane. After standing for two hours the solvent was evaporated, and the practically white residue crystallised from acetic acid. It separated as white needles. Yield 13·5 mg. M.P. 200–202° C. It is insoluble in cold or hot dilute caustic soda. An alcoholic solution with alcoholic ferric chloride gives no colour reaction.

Analysis.

4·452 mg. gave 8·140 mg. CO_2; 1·320 mg. H_2O.
3·415 mg. gave 5·530 mg. AgI.
Nitrogen test negative.
Found C = 49·87; H = 3·29; OCH_3 = 21·36.
$C_{14}H_4O_2Cl_3(OCH_3)_3$ requires C = 50·5; H = 3·22; OCH_3 = 23·02.

A mixture of 70 parts of trimethyl- and 30 parts dimethyl-concretin requires

C = 50·14; H = 3·1; OCH_3 = 20·9.

The product is insoluble in dilute potash, and gives no reaction with alcoholic ferric chloride.

Trichlor-ravenelin.

For purposes of comparison with concretin trichlor ravenelin was prepared.

We wish to thank Professor R. Robinson for the sample of ravenelin.

2 g. of ravenelin was dissolved in 400 c.c. nitrobenzene, and the whole saturated with chlorine in the cold. On standing overnight the nitrobenzene was distilled off in steam, the residue taken up in ether, dried, and recrystallised from a mixture of equal parts acetone and alcohol. A product of deep orange colour was obtained M.P. 195–197° C.

Analysis.

4·881 mg. gave 7·035 mg. CO_2; 0·560 mg. H_2O.
12·451 mg. gave 20·590 mg. AgCl.
Found C = 39·3; H = 1·27; Cl = 40·9.
$C_{14}H_5O_5Cl_5$ requires C = 39·0; H = 1·16; Cl = 41·2.

This pentachlor compound was dissolved in acetic acid and reduced with stannous chloride and hydrochloric acid. The whole was diluted with water, and extracted with ether. The ethereal solution was well washed with water, dried, and the product recovered from it recrystallised from methyl alcohol. Yellow

needles M.P. 234–237° C. were obtained. For analysis this was dried in high vacuum at 100° C.

Analysis.

5·011 mg. gave 8·610 mg. CO_2: 0·950 mg. H_2O.
6·971 mg. gave 8·080 mg. AgCl.
Found C = 46·86; H = 2·10; Cl = 28·67.
$C_{14}H_7O_5Cl_3$ requires C = 46·47; H = 1·94; Cl = 29·4.

Trichlor-ravenelin is soluble in warm sodium carbonate, and gives a brown-red solution with strong sulphuric acid.

Acetone Extract of Lichen.

After extraction with ether the lichen was air-dried and extracted by boiling with two litres of acetone for three hours. The acetone extract was filtered off, and the operation repeated with a further two litres of acetone. The hot acetone solutions on cooling deposited a grey powder which was collected. On concentration of the acetone liquors to a small bulk a further quantity of material was obtained. The whole was crystallised from dioxan from which it separated in the form of white needles: Weight = 15 g. It was identical in all respects with the white product (0·6 g.) obtained from the ether extract. It melted with decomposition at 267° C.

Analysis.

5·070 mg. gave 10·755 mg. CO_2; 1·500 mg. H_2O.
Found C = 57·85; H = 3·28.
$C_{18}H_{12}O_9$ requires C = 58·06; H = 3·22.
A methoxyl determination gave a negative result.

An alcoholic solution of the material gives with alcoholic ferric chloride a port-wine colour not destroyed by the addition of water. A sample of the product submitted to Professor Asahina was kindly confirmed by him as nor-stictic acid.

Penta-acetyl nor-stictic Acid.

0·5 g. of the product from the acetone extract was suspended in 10 c.c. acetic anhydride, and a few drops concentrated sulphuric acid added. After standing for some hours the whole was diluted with water, the white product collected, dried, and crystallised from a mixture of 6 c.c. acetone and 4 c.c. methyl alcohol M.P. 200° C.

Analysis.

4·753 mg. gave 9·750 mg. CO_2 and 1·770 mg. H_2O.
Found C = 55·95; H = 4·12.
$C_{18}H_9O_{10}(CH_3CO)_5$ requires C = 56·00; H = 4·00.

A sample of the product recrystallised from acetone melted at 206° C., and in this respect is identical with the penta-acetyl derivative prepared from nor-stictic acid isolated from *Cladonia subcariosa*. For this observation we are indebted to Professor Asahina.

Nor-stictic Acid Dianil.

0·5 g. of the product from the acetone extract was suspended in 100 c.c. of boiling 80 per cent. aqueous acetone; 0·5 c.c. of aniline was then added, and after boiling for one minute the whole was filtered. The filtrate on cooling deposited a mass of lozenge-shaped yellow crystals, Fig. 3, Pl. 17. These were collected: M.P. 260° C. with decomposition.

Analysis.

3·105 mg. gave 0·153 c.c. nitrogen at 26° C. and 739 mm.
Found N = 5·47.
$C_{18}H_{12}O_7(NC_6H_5)_2$ requires N = 5·36.

Alcohol Extract of the Lichen.

The lichen was dried after the acetone extraction, covered with alcohol, and boiled. The alcoholic extract was filtered, and the extraction repeated. The combined alcohol liquors were concentrated to 200 c.c., when a starch-like material was deposited from which it was not possible to obtain a crystalline product. After removal of this product the alcohol mother liquors were evaporated to dryness, the residue extracted with water, and the aqueous extract evaporated. The residue on spreading on a porous plate gave a brown almost solid material. This was boiled with ethyl alcohol, filtered from an insoluble fraction, and the alcohol solution evaporated to dryness. The residue was now triturated with cold methyl alcohol, the soluble fraction discarded, and the residue crystallised, first from hot methyl alcohol, and then from warm ethyl alcohol. A white crystalline product was thus obtained M.P. 165–166° C.

Mixed with mannitol there was no depression in melting point.

Analysis.

3·530 mg. gave 5·160 mg. CO_2 and 2·500 mg. H_2O.
Found C = 39·86; H = 7·87.
$C_6H_{14}O_6$ requires C = 39·55; H = 7·69.

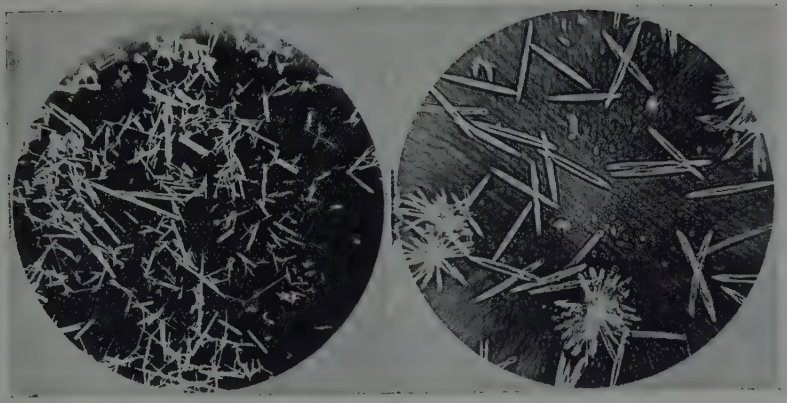


FIG. 1. FIG. 2.

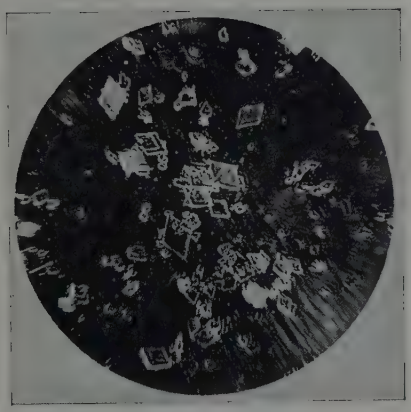


FIG. 3.

No. 53.

THE CHEMICAL CONSTITUENTS OF LICHENS FOUND IN IRELAND—*PARMELIA CONSPERSA* ACH.

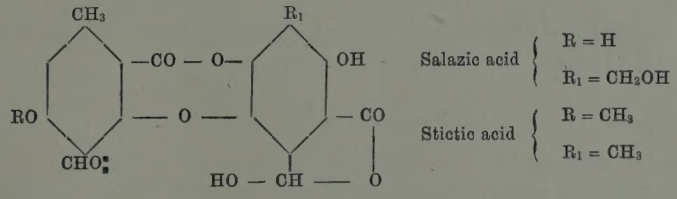
By MARGARET MOHAN, M.Sc., J. KEANE, Ph.D., and T. J. NOLAN, D.Sc.,
University College, Dublin.

[Read June 22. Published separately July 30, 1937.]

The lichen *Parmelia conspersa* (Ehrh.) Ach. has been examined by Zopf (Ann. der Chem., **297**, p. 282), who found in it usnic acid and salazic acid. The lichen was also examined by Hesse (J. Pr. Chem., **68**, p. 39), who also found usnic acid, but the colourless acid present he did not regard as salazic acid and called it conspersaic acid. In a later publication Hesse (J. Pr. Chem., **73**, p. 147) differentiates between salazic and conspersaic acids by their reactions towards alkalis. In a further publication Hesse (J. Pr. Chem., **83**, p. 81) persists in his view that the two acids are different, and rebuts Zopf's statement (Ann. der Chem., **340**, p. 298) that the acids are identical.

In recent years Asahina and Asano (Ber., **66**, p. 692) investigated *Parmelia conspersa* Ach. Var. *hypoclysta* Nyl, and in addition to usnic acid found salazic acid, which was identified by its physical characteristics and by the formation of red needles with alkali.

The lichen examined in the present investigation was collected on the maritime rocks in Howth on the promontory with a north-eastern aspect. It was identified by Dr. P. O'Connor, of the Natural History Division of the National Museum, as *Parmelia conspersa* Ach. To him we wish to express our thanks. In the lichen we identified the presence of usnic acid, but the main constituent was stictic acid; this observation was confirmed by analysis and by a comparison with stictic acid isolated from the lichen *Sticta pulmonaria*.



Experimental.

Ether Extract of Lichen.

The sample of air-dried lichen weighing 98 g. was left in contact with a litre of ether for 24 hours and the whole then filtered; this operation was repeated, and the combined extracts evaporated to dryness. A greenish-yellow solid weighing 2·5 g. was thus obtained.

After a preliminary treatment with petroleum ether, which removed only a small amount of an impure yellow product, the solid was boiled with two successive quantities of benzol (20 c.c.). From the benzol solution, on cooling, yellow crystals were obtained. This product on re-crystallisation melted at 204° C.; the following analysis shows it to be usnic acid:—

Analysis.

1·964 mg. gave 4·495 mg. CO_2 and 0·840 mg. H_2O.
Found C = 62·42. H = 4·75.
$C_{18}H_{16}O_7$ requires C = 62·8. H = 4·65.

The fraction insoluble in benzol was dissolved in boiling alcohol, and the alcoholic solution on cooling deposited a product which was identical with the main constituent of the acetone extract (*vide infra*).

Acetone Extract of Lichen.

The lichen after extraction with ether was air-dried and then extracted with four litres of boiling acetone. On evaporation, the acetone deposited 6 g. of a dark cream powder. This powder was boiled with 75 c.c. benzol, and on filtering, the benzol solution deposited crystals of usnic acid on cooling. The portion insoluble in benzol was treated successively with alcohol (75 c.c.) and acetone (75 c.c.); these solvents on cooling deposited small amounts of impure stictic acid. The residue insoluble in alcohol and acetone and which weighed 4·5 g. was crystallised from cyclohexanone, and gave a product melting with decomposition at 264° C.

Analysis.

5·047 mg. gave 10·890 mg. CO_2 and 1·750 mg. H_2O.
3·407 mg. gave 1·960 mg. AgI.
Found C = 58·85; H = 3·85; OCH_3 = 7·6.
$C_{18}H_{11}O_8 . OCH_3$ requires C = 59·07; H = 3·63; OCH_3 = 8·03.

The reactions of the product were identical in all respects with a sample of stictic acid isolated from *Sticta pulmonaria.*

Tetra-acetyl stictic Acid.

0·5 g. of stictic acid was suspended in 25 c.c. of acetic anhydride and a few drops of strong sulphuric acid added. On standing overnight the solution was diluted with water and the precipitated product crystallised from a mixture of benzol and ligroin; it melted at 232–233° C.

Analysis.

4·341 mg. gave 8·990 mg. CO_2 and 1·450 mg. H_2O.
2·701 mg. gave 1·090 mg. AgI.
Found C = 56·48; H = 3·71; OCH_3 = 5·32.
$C_{18}H_9O_9(OCH_3)(CO . CH_3)_4$ requires C = 56·65; H = 4·19; OCH_3 = 5·42.

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